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Title:
Intraspecific variation in responses to underwater anthropogenic noise

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A dissertation submitted to the University of Bristol in accordance with the requirements for award of the degree of Doctor of Philosophy in the Faculty of Life Sciences

August 2019

Word count: 32,960

Abstract

Anthropogenic noise is pervasive in terrestrial and aquatic environments, and recognised as a pollutant in national and international legislation. Extensive research in the last 15 years has shown effects of noise across taxa on both physiology and behaviour. However, the majority of that work has only considered the overall effects of noise, reporting responses as a mean cohort effect and ignoring the variation that exists within a species due to both intrinsic characteristics (e.g. size, sex and body condition) and extrinsic factors (e.g. prior experience, context and the existence of other stressors). In this thesis, I consider intraspecific variation in responses to anthropogenic noise, a greater focus on which will help avoid misinterpretations of effects and improve how we manage, monitor, model and mitigate the impacts of noise on wildlife. I start with a systematic and comprehensive review of the peer-reviewed literature pertaining to the causes and consequences of intraspecific variation in responses to anthropogenic noise (Chapter 1). In the first data chapter (Chapter 2), I examine the effects of pile-driving playback on the physiology and behaviour of Atlantic salmon (*Salmo salar*). I find no effects of pile-driving noise on any response measure, but develop methodology for use in later chapters. In Chapter 3, I begin assessing intraspecific variation in responses by determining the influence of body condition and habitat quality on noise impacts in the blue-green damselfish (*Chromis viridis*). Habitat quality had no effect on responses to noise, but individuals in poorer body condition performed worse than better-condition conspecifics in an anti-predator experiment. I then start to consider the influence of prior experience by testing the responses of *Cynotilapia zebroides*, an endemic cichlid fish, from high- and low-disturbance sites to noise exposure (Chapter 4). Fish from high-disturbance sites had a reduced sensitivity to motorboat noise compared to conspecifics from low-disturbance sites. Going a step further, I investigate the impact of chronic noise exposure within a generation using experimental manipulations to determine the potential for behavioural plasticity (Chapter 5). I found no changes in responses of *Stegastes nigricans* following a month-long manipulation of motorboat noise. However, chronic anthropogenic-noise exposure had effects at the community level. Comparing sites varying naturally in the level of motorboat disturbance, a number of species differed in their abundance (some were more prevalent, some less so, in high- vs low-disturbance sites). Some of these abundance differences were replicated following the month-long experimental manipulation of motorboat noise. Overall, my work emphasises the importance of considering variation in responses to noise if we are to understand fully and mitigate successfully this global pollutant.

Acknowledgments

First and foremost, I would like to thank Andy Radford and Steve Simpson for their years of support, encouragement, and expert tuition. It is without a shadow of a doubt that I would not have been able to get this far without them. I look back to the start of this PhD and the years before that and see how much progress I've made, and can say that it is all down to them. Thankfully, it's now safe to say I've habituated to the sight of the 'dreaded' red text scribbled all over manuscripts; from the en–dash to the em-dash, thank you for your tireless efforts.

Thank you to Ian Davies (Marine Scotland Science), my second co-supervisor, whose support before and during the early stages of the PhD was key in helping me along this journey. I learnt a huge amount during my time in at Marine Scotland, which was a very influential period in my development as a scientist. Along with Marine Scotland, I would not have even had the opportunity without funding supplied by the University of Bristol and the Natural Environment Research Council (NERC), so for that, thank you.

I would like to thank my close colleagues and collaborators that I have been very lucky to get to know over the past four years. The research and working environment they've helped create has made this journey even better. From the very early starts on Lizard Island where we stood on the beach debating who was to collect the boat from the mooring, to the tireless but special commutes to our field sites around Mo'orea, thank you. A massive overall thank you to the Swag Babes (I don't think anyone really knows what the acronym stands for anymore). Being part of such a great team has really made this last four years, it definitely would not have been the same without you all.

My family and friends deserve the utmost of gratitude; for the endless encouragement and emotional support during all the ups and downs. It's very easy to get held up on all the negative aspects and continually fall into the trap of imposter syndrome, but all your kind words and positivity about celebrating every little bit of success, no matter how small, was hugely beneficial. Finally, a very special mention to Genna Davies for your unremitting encouragement, dedication, support, love, wisdom, the list goes on...thank you. I would never have made it to the end if it weren't for your love and support, and for that, I can't thank you enough.

Author’s Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

Signed:

Date: 06.08.2019

Research articles published during the PhD

Bruintjes R, **Harding HR**, Bunce T, Birch F, Lister J, Spiga I, Benson T, Rossington K, Jones D, Tyler CR, et al. 2017. Shipbuilding docks as experimental systems for realistic assessments of anthropogenic stressors on marine organisms. BioSci. 67:853–859.

Bruintjes R, Simpson SD, **Harding H**, Bunce T, Benson T, Rossington K, Jones D. 2016. The impact of experimental impact pile driving on oxygen uptake in black seabream and plaice. Proc Meet Acoust. 27:010042 .

Gordon TAC, **Harding HR**, Clever FK, Davidson IK, Davison W, Montgomery DW, Weatherhead RC, Windsor FM, Armstrong JD, Bardonnet A, et al. 2018. Fishes in a changing world: learning from the past to promote sustainability of fish populations. J Fish Biol. 92:804–827.

Gordon TAC, **Harding HR**, Wong KE, Merchant ND, Meekan MG, McCormick MI, Radford AN, Simpson SD. 2018. Habitat degradation negatively affects auditory settlement behavior of coral reef fishes. Proc Natl Acad Sci USA. 115:5193–5198.

Harding HR, Gordon TAC, Eastcott E, Simpson SD, Radford AN. 2019. Causes and consequences of intraspecific variation in animal responses to anthropogenic noise. Behav Ecol. Online early. **This thesis: Chapter 1.**

Harding HR, Gordon TAC, Hsuan RE, Mackaness ACE, Radford AN, Simpson SD. 2018. Fish in habitats with higher motorboat disturbance show reduced sensitivity to motorboat noise. Biol Lett. 14:20180441. **This thesis: Chapter 3.**

McCormick MI, Allan BJM, **Harding H**, Simpson SD. 2018. Boat noise impacts risk assessment in a coral reef fish but effects depend on engine type. Sci Rep. 8:3847.

Roberts L, **Harding HR**, Voellmy I, Bruintjes R, Simpson SD, Radford AN, Breithaupt T, Elliot M. 2016. Exposure of benthic invertebrates to sediment vibration: from laboratory experiments to outdoor simulated pile-driving. Proc Meet Acoust. 27:010029.

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Chapter 1 – Causes and consequences of intraspecific variation in animal responses to anthropogenic noise

Contributions to the work

H.R.H. developed the initial ideas for the review with supervisors S.D.S. and A.N.R. H.R.H., with S.D.S., A.N.R., T.A.C.G. (University of Exeter PhD student working on how marine soundscapes are affected by global change) and E.E. (University of Bristol Masters student working on intraspecific variation in mongoose responses to noise), developed the overall structure and content. H.R.H. conducted the systematic literature review, with help from T.A.C.G., and conducted the qualitative analyses and effect-size calculations. H.R.H. wrote the initial manuscript with all authors contributing to produce the final version.

Sections 1.1 to 1.6 of this chapter have been published as:
Harding HR, Gordon TAC, Eastcott E, Simpson SD, Radford AN. 2019. Causes and consequences of intraspecific variation in animal responses to anthropogenic noise. *Behav. Ecol.* Online early. doi:10.1093/beheco/arz114.

1.1 ABSTRACT

Anthropogenic noise is a recognised global pollutant, affecting a wide range of non-human animals. However, most research considers only whether noise pollution has an impact, ignoring that individuals within a species or population exhibit substantial variation in responses to stress. Here, we first outline how intrinsic characteristics (e.g. body size, condition, sex and personality) and extrinsic factors (e.g. environmental context, repeated exposure, prior experience and multiple stressors) can affect responses to environmental stressors. We then present the results of a systematic search of the anthropogenic-noise literature, identifying papers that investigated intraspecific variation in the response of non-human animals to noise. This reveals that fewer than 10% of papers (51 of 589) examining impacts of noise test experimentally for intraspecific variation in responses; of those that do, over 75% report significant effects. We assess these existing studies to determine the current scope of research and findings to-date, and to provide suggestions for good practice in the design, implementation and reporting of robust experiments in this field. We close by explaining how understanding intraspecific variation in responses to anthropogenic noise is crucial for improving how we manage captive animals, monitor wild populations, model species responses, and mitigate effects of noise pollution on wildlife. Our aim is to stimulate greater knowledge and more effective management of the harmful consequences of this global pollutant.

1.2 INTRODUCTION

Human population growth, rapid urbanisation and infrastructure development, greater resource exploration and extraction, and the expansion of transportation networks have all contributed to the increased production of anthropogenic noise, altering terrestrial and aquatic soundscapes worldwide (Kight and Swaddle 2011; Shannon et al. 2015; Buxton et al. 2017). Many human activities generate noise within the hearing ranges of other animals, at sound levels above those found naturally and with different acoustic characteristics from abiotic and biotic sounds (Hildebrand 2009). Those man-made additions to the acoustic environment that contain little or no useful information and which have negative consequences on wildlife represent a well-recognised form of pollution. A wide variety of anthropogenic noise sources have been shown to affect invertebrate, fish, amphibian, bird and mammal behaviour (e.g. disrupting vocal communication, foraging, anti-predator responses and parental care), physiology (e.g. causing stress, hearing damage and immune-system impairment) and development (e.g. reducing growth and causing morphological malformations), with resulting fitness consequences (for recent reviews see: Morley

et al. 2014; Shannon et al. 2015; Kunc et al. 2016). However, most research has only considered whether noise pollution has an effect and the nature of its impact. Typically, empirical studies are inherently based on the assumption that conspecifics are ecologically equivalent, reporting responses as a mean cohort effect. Such a simplification ignores intraspecific (within-species) variation (Radford et al. 2016a).

Considerable variation exists between individuals of the same species for both intrinsic and extrinsic reasons, causing differences in the way that conspecifics look, behave and respond to natural selection pressures such as predation risk, food availability and novel environments (Bolnick et al. 2003). It is therefore inevitable that when presented with anthropogenic stressors, individuals from the same species will respond in different ways (Bolnick et al. 2011). These varied responses may define the difference between success and failure; the likelihood of mortality or the ability to emigrate, to adapt through genetic changes, or to respond via phenotypic plasticity (Engås et al. 1996; Höglund et al. 2008; Cripps et al. 2014). Intraspecific variation in responses can also have far-reaching impacts on the population dynamics, community structure and ecosystem function of entire groups of animals (Post et al. 2008; Rudman et al. 2015; Charette and Derry 2016; Des Roches et al. 2017). Indeed, in some cases intraspecific variation can have a greater influence than interspecific differences on overall community responses to environmental change (Crutsinger et al. 2006; Siefert and Ritchie 2016; Raffard et al. 2019). Furthermore, varied responses set the stage for future evolution, as the cohort of individuals capable of reproducing following an anthropogenic stress event defines the evolutionary potential of the post-disturbance population (Medina et al. 2007; Bijlsma and Loeschcke 2012). To consider only 'mean' responses to anthropogenic stressors is therefore to underappreciate the likely consequences of the disturbance; a lack of population-level impacts may be masking more subtle but important within-population changes. Conversely, consideration of intraspecific variation facilitates a more comprehensive understanding of the impacts of anthropogenic stressors on animals, the likely consequences for wider ecosystems, and the best management strategies to address these changes.

In this review, we begin by outlining the existence and importance of intraspecific variation in response to environmental stressors, and why its consideration with respect to anthropogenic noise is needed. We explain how variation arising from intrinsic characteristics (e.g. body size, body condition, sex and personality) and extrinsic factors (e.g. environmental context, repeated exposure, prior experience and multiple stressors) can affect responses in biological systems, and the consequences of such variation. We then report on a systematic review of the literature relating to

the impacts of anthropogenic noise on non-human animals. We provide a comprehensive list of experimental studies that have investigated intraspecific variation in responses to noise, and offer qualitative and quantitative summaries of the scope and findings of that research. Moreover, we draw on an assessment of those existing studies when making suggestions for best practice in designing and implementing robust experimental research that would benefit the field moving forwards. Finally, we explain how a greater focus on intraspecific variation in response to anthropogenic noise is crucial for improving the management of animals in captivity, monitoring the impacts on wild populations, modelling species responses, and mitigating the effects on wildlife. Our aim is to stimulate a greater understanding of the importance of intraspecific variation when determining both the impacts of anthropogenic noise and how best to mitigate this global pollutant.

1.3 THE EXISTENCE AND IMPORTANCE OF INTRASPECIFIC VARIATION

Intraspecific variation is caused by a range of intrinsic characteristics and extrinsic factors. Fundamentally, genotypic and epigenetic differences between individuals underpin intrinsic phenotypic characteristics (e.g. body size, body condition, sex and personality) that vary within a population (Skinner 2015). Individuals with different characteristics may respond differently to environmental and anthropogenic stressors in terms of both their behaviour and physiology (as discussed in detail below). These responses may be the consequence of, or be mediated by, differences in life-history trade-offs and strategies (Stearns 1992; Zera and Harshman 2001). Considerable intraspecific differences also arise due to extrinsic factors, including variation in the environmental context in which a stressor is experienced, repeated exposure to or prior experience of a stressor, and the presence and magnitude of multiple stressors. Many of these extrinsic factors (such as repeated exposure or prior experience) are likely underpinned by the flexibility in behavioural and/or physiological responses to sustained exposure to stressors; phenotypic plasticity is often the first line of defence when organisms are confronted with environmental change (Chevin et al. 2010; Wong and Candolin 2015). In this section, we describe several general mechanisms by which intraspecific variation can exert an influence on responses to environmental stressors and highlight the importance of considering this variation for an understanding of responses to anthropogenic disturbances such as noise. We do not provide an exhaustive list of potential characteristics and factors, but use illustrative examples where there is strong existing evidence for an influence.

1.3.1 Intrinsic characteristics

Variation in body size, which often scales with age (Sebens 1987), affects responses to environmental stressors due to fundamental differences in physiological mechanisms, morphology and behaviour (Spear et al. 1996; Ortiz-Santaliestra et al. 2006; Pörtner and Knust 2007). Size-dependent selection can drive changes in the demographic structure of populations (see Fig. 1.1a); for example, selective harvesting of larger individuals by commercial fishing fleets can cause shifts towards higher proportions of younger age classes among the spawning stock (Ottersen et al. 2006). In many taxa, body size correlates with fecundity (Shine 1988) and so stressors that truncate population size-structure may impact population reproductive potential and recruitment success, whilst also reducing the capacity to cope with further stress in variable environments (Kiffney and Clements 1996; Walther et al. 2002; Planque et al. 2010).

Conspecific individuals differ in body condition as a result of the heterogeneous nature of food availability and from inherent variation in routine metabolic rates (Metcalfe and Monaghan 2001; Killen et al. 2011). Variation in body condition results in considerable differences in behavioural and physiological functioning (Duckworth et al. 2001), and can therefore affect responses to environmental stress. Animals in poor condition with low energy reserves may display more risk-prone behaviours (Caraco et al. 1990), be unable to maintain optimal physiological functioning when challenged by a stressor, or fail to recover from additional environmental stresses (Sokolova et al. 2012; Sokolova 2013). Considering variation in body condition is important for the development of management and mitigation strategies to alleviate anthropogenic stress. For instance, during periods of reduced foraging opportunities or increased physiological stress, animals may be more at risk from anthropogenic stressors, and thus mitigation strategies become increasingly important at these times.

Sex-dependent effects occur in animals due to differences in morphology, biochemical processes and hormonal profiles (McClellan-Green et al. 2007; Palanza 2017). For example, if there are sex differences in baseline levels of stress-induced hormones, which influence individual responses to disturbances (Pottinger et al. 1996; Dalla et al. 2011), then males and females may respond differently to the same environmental stressor. In some species, sex-mediated responses to stress may be an adaptive mechanism associated with different energetic requirements (Afonso et al. 2003). Different effects of environmental stress on each sex may have profound population-level consequences, including with respect to sex ratios which may become altered by, for instance, sex-

specific mortality (Grüebler et al. 2008) or impacts on temperature-dependent sex determination (Parrott and Blunt 2005; Jensen et al. 2018). Potential impacts will be species-specific and related to mating strategy, but could include a decline in reproductive output affecting overall population viability (White et al. 2017; Jensen et al. 2018).

Intraspecific variation in animal personality—defined by a suite of behavioural traits consistent across time and environmental context—has been shown in multiple taxa (Sih et al. 2004; Réale et al. 2007). Personality co-varies with physiological and neuroendocrinological mechanisms, determining an individual's coping style; that is, how they deal behaviourally and physiologically with environmental stress (Carere et al. 2010). Personality type can affect how individuals perform in changing environments: in some systems, bold, fast-exploring, proactive individuals may do well in less-risky, stable environments, whereas slow-exploring, reactive behavioural types may perform better in high-risk environments and in situations of environmental change as they may have greater behavioural flexibility (Guillette et al. 2011; Sih et al. 2012). Maintenance of variation is important for adaptability to future environmental fluctuations (Dall et al. 2004; Sih et al. 2012). Furthermore, intraspecific variation in personality can have important implications for ecological processes, with variation in predator personalities shown to influence the composition of prey communities (Royauté and Pruitt 2015).

1.3.2 Extrinsic factors

The current environmental context, including food availability and predation risk, can affect behaviour exhibited by individual organisms (Lima and Dill 1990; Sih et al. 2004). Behavioural variability due to different environmental contexts reflects a trade-off between the risk from a stressor and the benefit gained from continuing a current activity (Lima and Dill 1990). For instance, many animals reduce their foraging during periods of increased predation risk (Clarke 1983; Lima and Dill 1990), and anthropogenic disturbances can influence predation risk (Chalfoun et al. 2002). Treating context as homogenous across studies compromises the quality of documented information about predicted responses to environmental stress.

Behavioural and physiological responses can change with repeated exposure to stressors (see Fig. 1.1b), across a range of taxa (Burger and Gochfeld 1999; Bejder et al. 2009). These modifications can also be transferred to offspring through epigenetic mechanisms (Dias and Ressler 2014). Moreover, anthropogenic stressors vary across time and space (Hildebrand 2009; Mekonnen

and Hoekstra 2015), meaning that individuals within a population or in different populations are likely to experience different conditions from one another. Variation in this prior experience can influence current responses, resulting in stronger effects due to sensitisation, or weaker effects due to increased tolerance or habituation (Bejder et al. 2009). Using data based on short-term responses from assays that do not link directly to fitness may therefore under- or over-estimate realised impacts on populations (Bejder et al. 2009).

Organisms are rarely exposed to stressors in isolation, due to the multitude of anthropogenic threats faced by animals worldwide; these threats include light and chemical pollution, changing climates, hypoxia, acidification of marine and freshwater systems, and habitat destruction and fragmentation (Millennium Ecosystem Assessment 2005; McBryan et al. 2013). The effects of multiple stressors can be additive or multiplicative, or one stressor can dominate another; additionally, interactions may be synergistic or antagonistic (see Fig. 1.1c) (Côté et al. 2016; Gunderson et al. 2016). Understanding responses to single stressors does not always allow realistic predictions of responses to multiple stressors (Darling and Côté 2008); populations may show no adverse effects to particular pollutants in isolation, but the addition of another stressor may cause a markedly different response (Relyea and Mills 2001) or even stress individuals beyond their physiological limit (Fasola et al. 2015).

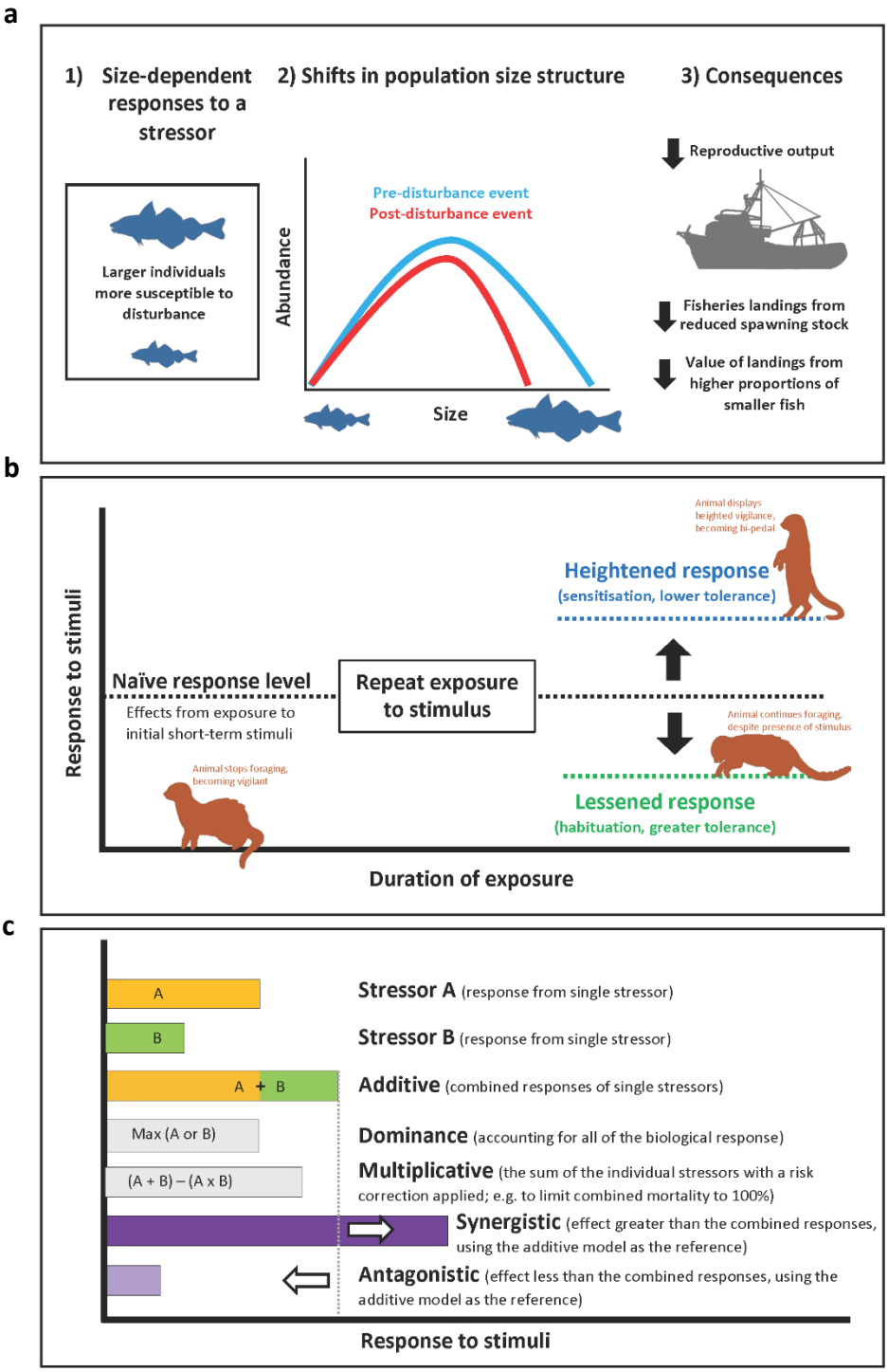

Figure 1.1. Importance of considering intraspecific variation in responses to environmental change. (a) Body size can affect responses to environmental stress with potential consequences for population-size structure and reproductive output, and thus implications for human economics and food security. (b) Prior experience of a stimulus may lead to a change in the level of response exhibited. (c) Multiple stressors can result in a variety of different response levels depending how the individual stressors interact (Côté et al. 2016). Images in figure drawn by WiseArt.net.

1.4 STATE OF KNOWLEDGE WITH RESPECT TO ANTHROPOGENIC NOISE

We performed a systematic search of the peer-reviewed literature that has investigated the impacts of anthropogenic noise on non-human animals (see Appendix A for methods), with three main aims. First, we identified the number and scope of studies examining intraspecific variation in response to anthropogenic noise. Second, we used the resulting comprehensive list of experimental studies to compare findings relating to different intrinsic characteristics and extrinsic factors. Finally, we drew on an assessment of those existing studies to make suggestions for best practice in the design and implementation of experimental research that would benefit the field moving forwards.

1.4.1 Research focus to-date

The body of literature investigating the effects of anthropogenic noise on non-human animals has increased rapidly in the last decade (Fig. 1.2; Shannon et al. 2015). The proportion of peer-reviewed studies considering intraspecific variation has also been growing, especially since 2013, although the absolute number still remains low (Fig. 1.2). It is possible (as in all research fields) that a publication bias exists towards papers that find an effect; there may be some that set out to test for intraspecific variation but, on finding no evidence, subsequently pooled results to report a general effect of noise. From our literature search, we identified 65 papers that have tested intraspecific variation in response to anthropogenic noise. These comprise 51 experimental studies (detailed in Table A.1, Appendix A) and 14 observational studies (Table A.2, Appendix A), representing 8.7% and 2.4% respectively of all papers published on anthropogenic noise that met our criteria. At present, the majority of noise studies testing intraspecific variation have considered extrinsic factors (n = 41, 71%), of which *repeated exposure* (n = 16) has been examined the most, followed by *environmental context* (n = 13)*, prior experience* (n = 8) and *multiple stressors* (n = 4). Intrinsic characteristics are less well-represented in our identified papers (n = 17; 29%); where a paper documents consideration of two or more sources of intraspecific variation (n = 7), they are included multiple times in this assessment. Among intrinsic characteristics, *sex* (n = 8) has been investigated the most often, followed by *body size/age* (n = 6), *body condition* (n = 2) and *personality* (n = 1).

The experimental studies conducted to-date span a broad taxonomic range and have considered a variety of response measures. *Fish* are the most well-documented taxa (n = 22, 42% of experimental studies investigating intraspecific variation), followed by *birds* (n = 14, 27%), *mammals* (n = 8, 15%; aquatic: n = 5, terrestrial: n = 3), *arthropods* (n = 5, 10%; aquatic: n = 3, terrestrial: n = 2)

and *amphibians* (n = 3, 6%); one study considered both *amphibians* and *arthropods*. Shannon *et al.* (2015) identified birds and marine mammals as by far the most-studied taxa in terms of noise impacts in general; the relative preponderance of fish studies on intraspecific variation likely reflects a recent upsurge in their consideration in anthropogenic-noise research (Kunc et al. 2016). In general, there is a strong taxonomic bias towards vertebrates, despite invertebrates making up 97% of known animals, having great ecosystem and commercial importance, and offering the opportunity for valuable experimental tractability (Morley et al. 2014). With regards to specific response measures, the majority of experimental noise studies considering intraspecific variation have focussed solely on *behavioural* responses (n = 37, 73%), compared to eight (16%) for *physiological* measures and six (12%) where *both* behaviour and physiology data have been collected. Only two studies (4%) have directly measured *fitness* impacts; while fitness estimates are often logistically more challenging to determine, they are what is ultimately required to assess population consequences.

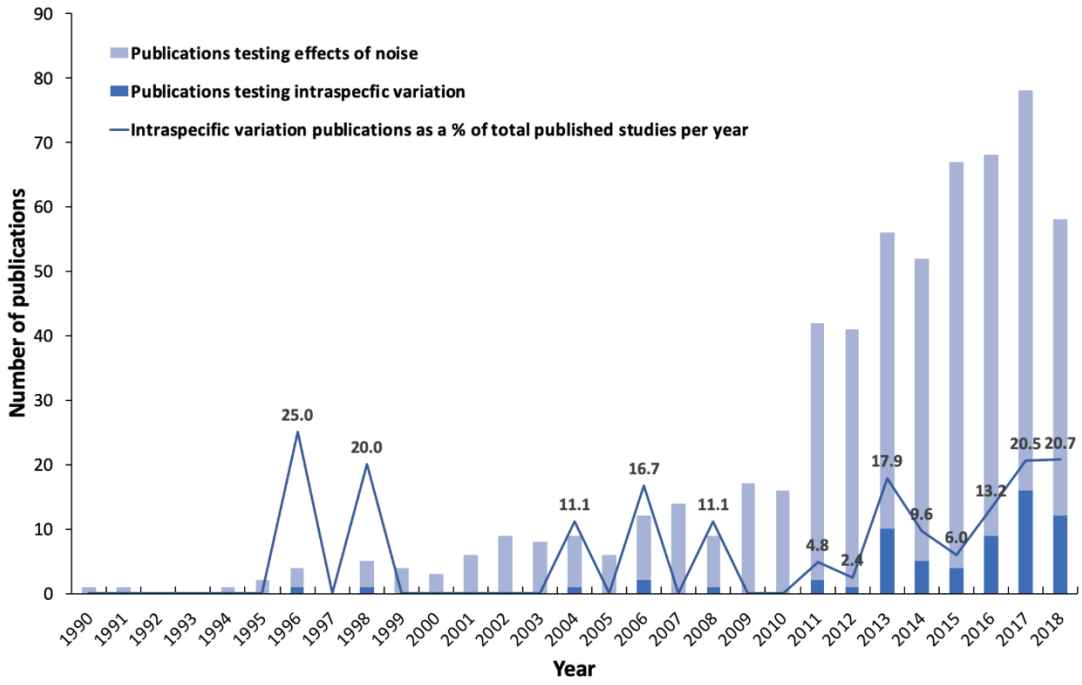

Figure 1.2. Number of peer-reviewed publications per year that have investigated the effects of anthropogenic noise, and intraspecific response variation, in non-human animals.

1.4.2 Current knowledge base

There are some qualitative differences in findings between different sources of intraspecific variation in the experimental work conducted to-date; any conclusions drawn at this stage need to be cautious, due to the small number of relevant studies in each case. Of the 58 measured aspects of intraspecific variation detailed in Table A.1, 44 (76%) are reported as having a significant effect on the response to anthropogenic noise. Overall, intrinsic characteristics (13 out of 17 cases; 76%) and extrinsic factors (31 out of 41; 76%) were equally likely to have a significant influence. However, at the level of specific characteristics and factors, there were considerable differences. All studies considering *body size* (n = 6) and *personality* (n = 1) reported significant effects on responses to noise, with *environmental context* and *repeated exposure* having a significant influence in 92% and 81% of studies, respectively. There is greater variation between studies considering each of *sex* (63%), *prior experience* (63%) and *body condition* (50%) as aspects of intraspecific variation. Only 25% of studies investigating *multiple stressors* reported an alteration in the effect of anthropogenic noise in the presence of an additional stressor.

We also considered, where possible, the standardised and composite effect sizes of intraspecific variation found in experimental anthropogenic-noise studies (Table A.1). Only rarely were the effect sizes for individual categories of interest or the intraspecific variation itself reported. We therefore attempted to calculate effect sizes ourselves, but this proved difficult due to a lack of relevant information. In the future, it would be useful if studies either included the means, standard errors/deviations and sample sizes or the raw data to allow for accurate calculation of effect sizes from more complex experimental designs (repeated measures), or if they reported explicitly the statistical tests comparing the categories in question; only 29% (15/51) of studies in Table A.1 provided even some of this information. Consideration of the composite effect sizes that we were able to calculate (n = 15) for different intrinsic characteristics and extrinsic factors indicated that no particular source of intraspecific variation causes an obviously greater magnitude in response differences to noise than any other (Fig. 1.3). A large composite effect size represents either a single characteristic (e.g. male or female) that is substantially more sensitive to noise than its equivalent opposite, or two or more characteristics that are affected by noise compared to the baseline/control conditions. In the four sources of intraspecific variation for which composite effect sizes could be calculated for more than one study, only one (*multiple stressors*) shows no overlap in confidence intervals, suggesting a substantial difference in response between studies. The effect sizes for the two studies assessing multiple stressor impacts of noise may be related to the use of different

response metrics (behavioural and physiological), which has been shown to affect the overall magnitude in response across the anthropogenic-noise literature (Cox et al. 2018). From the remaining three sources, all three (*prior experience*, *repeated exposure* and *context*) comprised studies where effect sizes overlapped with each other. Clearly, formal meta-analytic comparisons will only become possible with a greater number of suitable studies in the future.

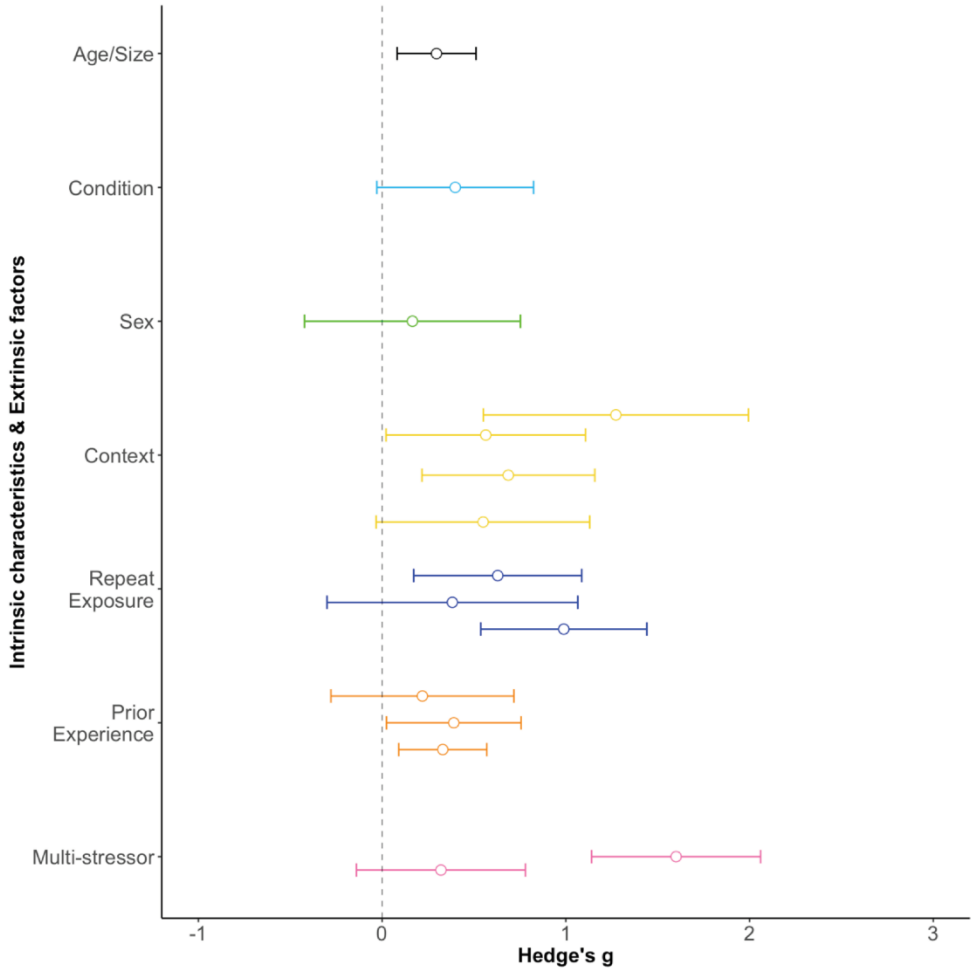

Figure 1.3. Standardised effect sizes (Hedge's *g*) for experimental studies in Table A.1 (calculated where possible and using illustrative examples where studies present more than one response metric) for different sources of intraspecific variation. Points and associated error bars represent composite standardised effect sizes (CES) and associated 95% confidence intervals from individual studies. CES were calculated in the following way: the weight of each group per study (1/variance of the effect size) was determined, and subsequently multiplied by the individual effect size (ES*weight); the composite effect size was then determined by dividing the sum of the effect size*weight by the sum of the weights. 95% confidence intervals were calculated with the following equation (CI = CES $\pm$ $(1.96(\sqrt{(1/\sum weights)}))$). All CES are presented as positive integers to enable illustrative comparisons across studies.

1.4.3 Future experimental studies

To improve our understanding of intraspecific variation in responses to noise, more and robust experimental tests are required. We suggest that a series of key decisions can aid the design and implementation of such tests; these are not mutually exclusive. Some of these decisions are broadly applicable to most, if not all, research fields and are frequently discussed. For instance, consideration of the relative advantages and disadvantages of captive vs field-based work, the need for suitable controls and sample sizes, and avoidance of pseudoreplication. We outline the importance of these fundamental concepts in Table A.3 (Appendix A), highlighting existing examples of good practice from experimental papers on intraspecific variation in noise responses. Decisions that have more specific relevance to the study of anthropogenic noise are described in detail below.

Rigorous anthropogenic-noise research needs to involve suitable acoustic measurements, including consideration of what is known about the hearing thresholds of the study species. Full characterisation of the sound field is required (rather than just the reporting of single decibel values) and should be presented in the appropriate domain for the species in question (Francis and Barber 2013; McKenna et al. 2016; Nedelec et al. 2016a). For aquatic studies on fish and invertebrates, this includes reporting acoustic metrics in both particle-motion and sound-pressure domains (Nedelec et al. 2016a); for terrestrial studies, the correct frequency weighting for the taxa needs to be applied (McKenna et al. 2016). Of studies in Table A.1, only 62% clearly do this (11 out of 25 fish and aquatic invertebrate studies; 17 out of 20 terrestrial studies). It has been suggested that sound measurements be recorded with both a Z-frequency weighting (flat response) and a weighting more appropriate for the study species (Francis and Barber 2013); only two examples of this approach exist in Table A.1 (LaZerte et al. 2016, 2017). Determining the best sound-characterisation approach should ideally be informed by knowledge of the hearing range of the species being studied; in some species at least, there can be ontogenetic changes in hearing thresholds (Kenyon 1996; Wright et al. 2011). However, care needs to be taken when using published hearing thresholds; for example, the validity of many published fish-hearing measurements has recently been called into question (Hawkins et al. 2015).

In addition to clear and detailed reporting of acoustic metrics, it is important to consider the advantages and disadvantages of using loudspeaker playback (the methodology employed in 43 of the studies in Table A.1) vs real noise sources (nine studies in Table A.1) for experiments on the impacts of anthropogenic noise. Loudspeaker playback enables isolation of noise as the stressor,

free from visual disturbances and other potential confounds (e.g. wake effects from passing boats or ships). However, use of loudspeakers results in sound fields that can differ considerably from those in real-world situations (Okumura et al. 2002; Quadros et al. 2014; Slabbekoorn 2016). Additionally, loudspeakers tend to provide only a point sound-source which is unrealistic for many types of anthropogenic noise, including vehicle traffic; this can be overcome with the use of multiple loudspeakers to create, for example, a phantom road (see McClure et al. 2013). To provide acoustic validity, real-noise sources are required but this presents a number of challenges. First, it is often logistically difficult to obtain access to real noise sources. Second, robust experimental design requires multiple exemplars of the noise source to minimise pseudoreplication (Kroodsma et al. 2001). The latter is particularly problematic when testing for impacts of noise in aquatic environments (e.g. commercial shipping or pile-driving), however motorboats offer the opportunity for well-controlled experiments (Simpson et al. 2016a). Whilst captive experiments are constrained to using loudspeaker playback, large-scale mesocosm and field-based studies allow the potential use of both acoustic methods, and therefore facilitate a beneficial comparison. Using both methods allows a demonstration of the effect of an actual human activity whilst also assessing the importance of the noise component alone, but there is only one study in Table A.1 that combines the use of both loudspeaker playback and real noise sources (Harding et al. 2018).

Most published work investigating intraspecific variation in anthropogenic-noise effects has focussed on either behavioural and/or physiological responses (see 'Research focus to-date'). So, the range of response measures could be profitably broadened, especially given the rapid advancement in genetic-sequencing technologies and reductions in processing costs (Connon et al. 2018). It is increasingly possible to use methods such as Restriction site-Associated DNA sequencing to explore genotypic variation (Paris et al. 2015), and transcriptomics to reveal the genetic effects of stressors (Pespeni et al. 2013), as well as determine potential plasticity and evolutionary adaptability (Munday et al. 2013); the use of these methods is in its infancy in anthropogenic-noise studies (see Chen et al. 2018 for an example). Ultimately, though, a full understanding of population and ecosystem consequences will require consideration of impacts on individual fitness. Ideally studies would measure these directly rather than extrapolating from, for instance, short-term behavioural and physiological responses. Clearly measuring fitness is logistically challenging, but Casper *et al.* (2013) and Potvin and Macdougall-Shackleton (2015) have demonstrated that it can be feasible with respect to intraspecific variation in response to anthropogenic noise.

1.5 APPLICATIONS FOR CAPTIVE AND WILD ANIMAL POPULATIONS

We believe that improving current understanding of intraspecific variation in responses to anthropogenic noise will increase our capability to manage captive animals effectively, monitor impacts on wild populations, model species responses, and mitigate the effects of noise pollution. Below, we provide specific suggestions as to how and why increasing our understanding can be valuable in each of these areas.

1.5.1 Management

Incorporating intraspecific variation in noise responses into the management of captive animals could provide benefits to welfare, food productivity and experimental control in research studies. Captive systems are often inherently noisy (for example, transportation and construction noise in zoos raise ambient sound levels, as do pumps and aerators in aquaculture facilities; Bart et al. 2001; Shepherdson et al. 2004), but can potentially be quietened by noise-reduction techniques (Davidson et al. 2007). Elevated noise in captivity can cause stress and negatively affect growth, condition and survival (Banner and Hyatt 1973; Shepherdson et al. 2004; Anderson et al. 2011). However, the effects of noise exposure may differ between cohorts or life-stages; for instance, animals may be especially vulnerable early in life (Davidson et al. 2007, 2009; de Soto et al. 2013; Nedelec et al. 2014). It is widely accepted that the feeding regimes needed to meet the energy and nutrient requirements of growing animals will change with age, and so diets are prepared accordingly for captive animals (Gardner et al. 1988; Wecke and Liebert 2013). If there are age-specific responses to noise, then tailoring noise-reduction management techniques to particular life-stages may similarly be beneficial to animal welfare, growth rates and productivity; in aquaculture, this could arise through, for instance, a better feed-conversion efficiency (Davidson et al. 2007). Moreover, acoustic noise in captive research systems should be minimised in order to remove unwanted statistical variation, potential confounding factors and possible biases in response data (Sabet et al. 2016a). Importantly, if intraspecific variation in response to noise exists, individuals from more or less susceptible cohorts should be split evenly between different experimental treatments to avoid biases.

1.5.2 Monitoring

Considering intraspecific variation is crucial when monitoring the responses of wild animal populations to anthropogenic noise. If intrinsic characteristics cause undetected or unconsidered response variation, then population assessments and predictions about resilience to environmental stress may be misleading (Mimura et al. 2017). For instance, European eels (*Anguilla anguilla*) in poorer body condition were shown to be more affected than those in better condition by ship noise (Purser et al. 2016). Depending on when impact assessments are made, noise responses may appear more or less severe as a result of temporal fluctuations in body condition within populations (Brosset et al. 2015). Studies focusing on population averages may also provide inaccurate impact assessments when responses to noise change with repeated exposure or prior experience (Radford et al. 2016b; Harding et al. 2018). Monitoring populations near to and far from human activities would allow cohorts with varying degrees of noise exposure to be assessed (Harding et al. 2018), but in doing so it is important to control for habitat type and other anthropogenic disturbances, to avoid potential confounds and isolate noise as the stressor (Francis et al. 2012). Additionally, incorporating long-term acoustic-monitoring data, such as those from the Ocean Noise Reference Station Network (Haver et al. 2018), into Environmental Impact Assessments (EIAs) could allow for consideration of prior experience and how exposure to different noise types can lead to specific or generalised response changes (Radford et al. 2016b). Including context-based and cumulative-impact evaluations into EIAs and monitoring could further reduce uncertainty when predicting behavioural and fitness effects (Ellison et al. 2012; Nowacek et al. 2015; Harris et al. 2018).

Interactions between species are highly flexible and can be influenced by intraspecific variation (Pruitt et al. 2012; Lichtenstein et al. 2016). For example, variation in the levels of an aggressive phenotype in a population of *Anelosimus studiosus* spiders was shown to alter relationships with heterospecifics and affect reproductive performance (Pruitt et al. 2012). Incorporating intraspecific variation of this nature may prove important for accurately determining the impact of noise on interactions between species—both antagonistic/conflict relationships (Simpson et al. 2016a; Nedelec et al. 2017a) and those of a more cooperative or mutualistic nature (Nedelec et al. 2017b)—and at the community level (Moran et al. 2016). In a hypothetical example, whilst a given predator–prey relationship may appear to be affected by noise in favour of one party (Simpson et al. 2016a), repeated exposure may adjust the balance of the relationship if, say, the prey becomes more tolerant and the predator continues to display its original response. Identifying the potential impacts of anthropogenic noise on the functional diversity of community assemblages

(type, range and abundance of organismal traits in a community; Díaz et al. 2007) will develop greater insight into how fundamental ecological processes and ecosystem stability may be affected. Predictions about impacts on functional diversity would be improved by considering intraspecific variation (Cianciaruso et al. 2009) because there can be differences in functional roles depending on such intrinsic characteristics as size and age (Bonaldo and Bellwood 2008). Intraspecific variation can have greater effects on indirect ecological interactions than removal or replacement of a species (Des Roches et al. 2017), and influence the strength of trophic cascades in a community (Post et al. 2008).

1.5.3 Modelling

Modelling species responses to anthropogenic noise would likely be improved by inclusion of intraspecific variation. Predictive modelling can be used for EIAs and for projecting future species distributions and understanding functional and community-level changes (Chevin et al. 2010; Rossington et al. 2013). Empirical investigations of physiological and behavioural responses are valuable for parameterising both population-persistence models and individual- and trait-based models, and for interpreting presence/absence and abundance data in combination with environmental variables in species-distribution models (SDMs) (Chevin et al. 2010; Koenigstein et al. 2016). Failure to capture intraspecific variation in baseline parameters may artificially reduce model uncertainty, but will also reduce the value of predictions when deciding mitigation measures and developing management strategies. Mechanistic population models can include multiple size/age classes and variation in phenotypic traits, while individual-based models can be expanded to include genotype (Moran et al. 2016). For example, including tree growth data as a measure of intraspecific variation improved the quality of pine tree distribution models relative to models that treated all trees as equal, and produced different future predictions as a result (Oney et al. 2013). Indeed, incorporating intraspecific variation into SDMs in this manner has been shown to alter predicted responses of species to environmental change in several cases (Valladares et al. 2014), and is therefore likely also to be important when modelling responses to anthropogenic noise.

1.5.4 Mitigation

Strategies for mitigating the impacts of anthropogenic noise should include the identification of traits that make particular individuals within a population especially vulnerable. For example, male natterjack toads (*Epidalea calamita*) exhibit high site-fidelity during breeding seasons, as they

remain located at a particular pond from which they call to attract free-ranging females (Sinsch 1992). In a hypothetical local noise-pollution scenario, mobile females might move out of a noisy area to nearby quieter habitat, but site-attached males may be unable to do so, with potentially important consequences for population demography and implications for the management of noise (Husté et al. 2006). Using only knowledge of short-term responses may also have detrimental consequences if, for instance, individuals habituate to stimuli over time and can compensate for initial impacts; mitigation measures required initially to avoid acute effects on survival (Simpson et al. 2016a) might subsequently become unnecessary, and their continued implementation could be conservative. By contrast, there may be declines in growth and fitness consequences for individuals inhabiting areas exposed to chronic noise, even if no acute changes in behaviour or physiology were initially displayed (Slabbekoorn et al. 2010).

In general, there is a need to determine both the spatial and temporal scale of noise impact, including the establishment of noise-exposure threshold levels (those at which different biological responses are predicted; Farcas et al. 2016). Incorporation of intraspecific variation into these threshold levels will improve their accuracy; the result might then be multiple spatial zones where different subsets of a population are likely to be affected. Additionally, impacts of noise during the breeding season might be especially detrimental, due to the increased fitness costs associated with reproductive failure (Nedelec et al. 2017a), and so noise mitigation during this period might have a greater population-level impact than at other times of year. As an existing conservation example, there is season-specific legislation on noise pollution with respect to marine mammal movement and behavioural patterns (Merchant et al. 2018; Pine et al. 2019). For instance, the *Be Whale Wise* regulations are a set of guidelines, developed collaboratively by government agencies, non-profit organisations and local stakeholders, for boat users in the Salish Sea (USA & Canada). These guidelines instruct users to take extra care to reduce vessel noise in the area between May and September, which is the breeding and pupping seasons for marine mammals (Be Whale Wise 2019). Overall, the aim should be to use information about intraspecific variation in noise responses to enhance the effectiveness of mitigation strategies.

1.6 CONCLUSIONS

Over the last 10–15 years, a rapidly burgeoning literature has provided substantial evidence that non-human animals from a wide range of taxa and ecosystems are detrimentally affected by anthropogenic noise (Morley et al. 2014; Shannon et al. 2015; Kunc et al. 2016). While there is

undoubted value in continuing to extend the geographic and taxonomic range of sampling (Shannon et al. 2015), there is also a need to expand the scope of studies. We argue that including greater consideration of intraspecific variation in responses would represent a profitable and important expansion in this regard. Doing so will generate a more complete and realistic understanding across all levels of biological organisation, helping to prevent misinterpretations that can lead to over- or under-estimation of the impacts of noise exposure. While it is well-recognised that care should be taken when extrapolating results between species, as responses may differ substantially (Shannon et al. 2015; Kunc et al. 2016), similar caution should be applied within species. Moreover, inclusion of intraspecific response variation in studies will enable better translation into suitable and effective management recommendations and actions. With continued urbanisation, energy-generation and transport-network expansion in a range of different ecosystems, it is likely that anthropogenic noise will become ever more prevalent across the globe. To understand its true impact and to mitigate its effects, we must pay careful attention to the variation that exists within populations and species.

1.7 FISHES IN A NOISY WORLD

The four data chapters in this thesis focus on how underwater noise pollution can affect animal responses, using a number of fish species as model organisms. There are an estimated 33,000+ species of fishes worldwide (FishBase; www.fishbase.in), occupying freshwater, marine and transitional waters. Fishes are fundamental components of aquatic ecological systems, performing a diverse array of ecological functions, which are crucial in maintaining resilience, food-web dynamics and nutrient recycling (Holmlund and Hammer 1999; Hughes et al. 2005; Allgeier et al. 2016). These supporting services directly contribute and maintain a healthy ecosystem from which human societies benefit. For instance, in 2016, wild capture fisheries and aquaculture provided work for approximately 60 million people around the world (FAO 2018); and with annual global marine-fisheries landings estimated at around 100 billion USD, they are of huge economic importance (Lam et al. 2016). Beyond the direct economic benefits, fishes represent an important food source for billions of people; 3.2 billion people get approximately 20% of their average per capita intake of animal protein from fish, 50% in some countries and small-island nations (FAO 2018). In addition to direct human consumption, fishes caught from the wild are used as a food source for livestock and aquaculture production (Lynch et al. 2016). Economic and provisioning services aside, there are a number of vital non-material services provided by aquatic ecosystems. Cultures hold many non-material values with aquatic environments, including a sense of place and spiritual connections with species, as well as an overall sense of happiness directly related with the health of the environment

(Hewitt et al. 2004; Millennium Ecosystem Assessment 2005; Russell et al. 2013; Pecl et al. 2017). Therefore, understanding and mitigating anthropogenic impacts on fish populations and the ecosystems they are part of is vital in protecting the crucial goods and services they provide.

Underwater noise pollution is generated from a variety of sources, including pile-driving, sonar, seismic arrays, commercial shipping and recreational boating (Hildebrand 2009; Sertlek et al. 2019). The majority of these sources are typically found in coastal regions, which have seen unprecedented increases in such activities since the mid-20th Century and are predicted to continue to rise due to the rapidly growing human population (Davenport and Davenport 2006; Neumann et al. 2015). Between 1950 and 2007, it has been estimated that ambient sound levels in the open ocean increased by 3.3 dB per decade (Frisk 2012), although the exact level will vary between locations. Further, global maritime traffic is expected to increase by between 240% and 1,200% by 2050 (Sardain et al. 2019), and recreational boat use on the Great Barrier Reef (GBR) is forecast to increase dramatically over the next few decades (Great Barrier Reef Marine Park Authority 2014). Marine fauna, including fishes, are therefore likely to encounter acoustic disturbances from human activities ever more frequently.

Underwater anthropogenic noise has been shown to affect fishes in a multitude of ways; for detailed reviews, see (Slabbekoorn et al. 2010; Kunc et al. 2016; Popper and Hawkins 2019). High intensity, impulsive noise can cause mortality and physical injuries, including ruptured swim bladders and haematomas (Halvorsen et al. 2012). Exposure to noise pollution can result in hearing threshold shifts (Smith et al. 2004). In addition, noise exposure can activate the stress axis, cause declines in growth rates and abnormalities in developing larvae (de Soto et al. 2013; Nedelec et al. 2015; Simpson et al. 2015). Further from the noise source, where the sound is quieter, behavioural effects are the most widespread and likely impacts. Experiments have shown changes in, for example, foraging ability, orientation, parental care, aggression, swimming patterns and shoal structure (Bruintjes and Radford 2013; Holles et al. 2013; Voellmy et al. 2014a; Neo et al. 2015; Sabet et al. 2015; Herbert-Read et al. 2017; Nedelec et al. 2017a).

The majority of research investigating the impacts of noise have focussed on mean responses of populations. Whilst there are a few recent examples of studies considering intraspecific variation of responses to noise with respect to fishes (Purser et al. 2016; Neo et al. 2018), work considering the influence of intrinsic characteristics (e.g. body condition) and extrinsic factors (e.g. prior experience, context) remains scarce (see table A.1, Appendix A). Incorporating intraspecific variation

is vital for a complete understanding and to avoid misinterpretations of the impacts of noise exposure. Addressing this knowledge gap would enable better management of captive fishes, monitoring of wild populations, modelling species responses to noise, and mitigating impacts (see earlier for details). Much of the existing work on the impacts of anthropogenic noise on fish has focussed on single-species responses, but there are starting to be studies investigating how noise affects interactions between species (e.g. predator–prey dynamics and cleaner–client mutualisms; Sabet et al. 2015; Simpson et al. 2016a; Nedelec et al. 2017b; McCormick et al. 2018a). However, to understand fully ecosystem-level responses we need to determine the effects on whole communities; whilst there are a few examples of this approach in terrestrial systems (Francis et al. 2009; Herrera-Montes and Aide 2011), similar research is needed with respect to underwater noise pollution. Identifying how communities respond to noise would enable more-developed mitigation strategies that focus on the weakest links, an improved understanding of how functional roles may change within an ecosystem, and the resilience of habitats in an increasingly noisy world.

1.8 THESIS AIMS

My thesis aims to further our understanding of intraspecific variation of responses to anthropogenic noise. Specifically, how such factors as body condition, habitat quality and acoustic history affect responses of fish to additional noise. The four data chapters represent a methodological progression from tank-based experiments using loudspeaker playback, to consideration of the responses of captive fish to real-noise sources, and culminating in the measurement of responses of free-swimming fish to real motorboats (i.e. full ecological and acoustic validity). An additional chapter development concerns the level of biological organisation, building from single-species responses to interspecific interactions and community-level impacts.

Chapter 2 uses loudspeaker playback in a laboratory setting to investigate the effects of pile-driving noise on Atlantic salmon (*Salmo salar*) behaviour and physiology, a topic of concern for many stakeholder groups and my CASE partner (Marine Scotland Science). To date, a large proportion of the work on underwater noise has been conducted in laboratory conditions or in field-based experiments using loudspeaker playback. This represents an important building block in fully understanding responses to anthropogenic noise, maximising experimental control and isolating noise as the stressor. In this chapter, I adapt existing methods for use with larger species, and refine them for use in subsequent chapters. The work therefore represented an important developmental

stage in the thesis, as well as allowing close collaboration with my CASE partner and a greater understanding of the needs of end-users for scientific research.

Chapter 3 is the first field experiment of the thesis, where I use real-noise sources for acoustic validity but wild-caught fish (*Chromis viridis*) in open-water conditions to test behavioural and physiological response measures. Importantly, this was also the first chapter where I moved beyond asking whether there is simply an effect of noise to testing variation in responses within a population. First, I was interested in whether body condition can affect responses to noise; responses of fish of relatively good and poor condition were compared. Second, responses of fish from degraded and healthy host corals were compared to test how habitat quality influences the impact of motorboat noise.

Understanding how the acoustic history of fish populations may alter current responses to noise is crucial and underpins management and mitigation strategies. In Chapter 4, I begin to address this issue by comparing the responses of an endemic Lake Malaŵi cichlid, *Cynotilapia zebroides*, from areas with different prior experiences of noise exposure using pre-established spatial variability in motorboat traffic. This chapter was field-based and used both real motorboats and loudspeaker playback to test physiological and behavioural responses to noise.

To develop insight into the potential mechanisms through which acoustic history can alter population responses, it is important to monitor and test the same individuals before and after chronic exposure to noise. Chapter 5 aimed to address this through a month-long experimental manipulation of the noise levels at natural sites in the lagoon surrounding the island of Mo'orea, French Polynesia. Real motorboats were used and behavioural responses (including intra- and interspecific interactions) of free-swimming fish (*Stegastes nigricans*) were determined. Moreover, community-level responses to chronic motorboat disturbance were assessed.

I finish the thesis with a discussion about my overall findings from each chapter, and then posit areas for further research stemming from both individual chapters and considering the field of anthropogenic noise more broadly. Finally, I conclude with a forward thinking perspective on the mitigation of noise produced from small motorboats, and discuss how emerging technologies may enable effective mitigation.

Chapter 2 – The effects of pile-driving playback on the behaviour and physiology of adult Atlantic salmon

Contributions to the work

H.R.H. conceived the research with supervisors I.D. (CASE partner—Marine Scotland), A.N.R. and S.D.S.; H.R.H., A.N.R. and S.D.S. designed the experiments; H.R.H. conducted the experiments and analyses, and interpreted the results, advised by A.N.R. and S.D.S.; H.R.H. drafted the chapter, with comments provided by I.D., A.N.R. and S.D.S.

Elements of this chapter have been published as an official report for Marine Scotland:
Harding HR, Davies I, Radford AN, Simpson SD. 2016. Part 2: The impact of pile-driving playback on the behaviour and physiology of Atlantic salmon (*Salmo salar*). Marine Scotland Science- publication. Available at: www.gov.scot/publications/measurement-hearing-atlantic-salmon-salmo-salar.

2.1 ABSTRACT

Anthropogenic noise pervades biomes around the globe, impacting both terrestrial and aquatic fauna. The continued development of marine renewable energy devices in Scottish coastal waters is a key step in the UK's commitment to clean, low-carbon energy. Unwanted noise associated with the construction of such devices, particularly pile-driving, is of concern due to potential effects on marine fauna. The two migratory phases of Atlantic salmon (*Salmo salar*) are likely to bring them into contact with areas of development. We used well-controlled tank-based experiments to test the potential effect of pile-driving playback on the behaviour and physiology of adult Atlantic salmon, whilst characterising the acoustic stimulus in both the particle-motion and sound-pressure domains. We found no evidence of a startle response or an effect on swimming speed at the onset of noise exposure. We also observed no avoidance behaviour associated with pile-driving playback nor any increase in oxygen consumption compared to control playback. This study provides an important stepping stone in the development of a comprehensive understanding of pile-driving impacts on organisms.

2.2 INTRODUCTION

Anthropogenic noise is widespread in aquatic environments and is increasing in prevalence spatially and temporally (Hildebrand 2005). Aquatic noise-generating activities include high-intensity air guns used for seismic exploration, chronic low-frequency noise from shipping, and noise produced during construction and operation of marine infra-structure, including energy installations. Some noises are produced deliberately, while others are by-products of the activities (Popper 2003; Hildebrand 2005; Slabbekoorn et al. 2010; Normandeau Associates 2012). The noise produced during such activities is very different to sounds that typically arise from natural sources (Hildebrand 2009; Radford et al. 2014). Due to concerns about the potential impact of additional noise on marine fauna, the introduction of acoustic energy from anthropogenic sources into the sea has been included as a quality descriptor in the European Commission Marine Strategy Framework Directive (The European Parliament and the Council of the European Union 2008), providing a statutory responsibility for member states to monitor and manage sound levels. It is therefore imperative that we increase our understanding of the potential impacts of underwater noise pollution through empirical investigations.

To date, anthropogenic noise has been shown to have a range of effects on marine organisms (Shannon et al. 2015; Kunc et al. 2016). Near to a strong impulsive sound source, physical injuries and mortality can occur in some species (Halvorsen et al. 2012). Further from the source, anthropogenic noise can alter the physiology of individuals without causing physical damage, sometimes resulting in stress and shifts in hearing thresholds (Smith et al. 2004; Wysocki et al. 2006; Slabbekoorn et al. 2010; Simpson et al. 2015). Furthest from the sound source, the most likely impacts are behavioural, which may be the most detrimental for populations due to the extended spatial scales over which impacts can occur (Normandeau Associates 2012; Simpson et al. 2015). Behavioural changes due to anthropogenic noise include those relating to foraging, communication, and anti-predator responses (Wysocki et al. 2006; Slabbekoorn et al. 2010; Purser and Radford 2011; Simpson et al. 2016a).

The continued development of Marine Renewable Energy Devices in Scottish coastal waters is recognised as an important step in the UK's drive for clean, low-carbon energy. Consequently, there are likely to be increased interactions between construction and native fish, including Atlantic salmon (*Salmo salar*). Atlantic salmon have been shown to detect pure tones below 380 Hz (Hawkins and Johnstone 1978), coinciding with the dominant frequencies produced during pile-driving operations (Bailey et al. 2010; Hawkins et al. 2015). The construction noise from offshore windfarms, therefore, has the potential to affect the two migratory stages of the Atlantic salmon life cycle: juveniles (smolt) leaving the rivers for the first time and adults returning to natal rivers after one or more years at sea. For adults, there is a concern that impact pile-driving in coastal regions may delay or prevent migration to natal rivers, with potential consequences for spawning. However, empirical investigations of the behavioural responses of Atlantic salmon to anthropogenic acoustic stimuli are lacking.

In this study, we adapted established laboratory-based experiments using loudspeaker playback (Simpson et al. 2015) to consider the potential impact of pile-driving noise on adult Atlantic salmon behaviour and physiology. This approach cannot result in a full natural repertoire of behavioural responses, and it does not fully replicate natural sounds due to reflections and reverberations within enclosed arenas (Okumura et al. 2002; Slabbekoorn 2016). However, it does enable the tight control of potential confounding variables and isolation of the effects of the acoustic stimulus when measuring a subset of potential behavioural and physiological responses (Bruintjes and Radford 2014; Simpson et al. 2015; Slabbekoorn 2016). We investigated the

behavioural and physiological impact of the additional noise of pile-driving playback compared to ambient-sound playback. From a behavioural perspective, we determined whether Atlantic salmon display immediate startle reactions and/or changes in swimming speed, and tested for subsequent avoidance behaviour in response to pile-driving noise. From a physiological perspective, we assessed whether pile-driving noise causes an increase in oxygen consumption.

2.3 METHODS

2.3.1 Ethics statement

All behavioural and physiological experimental procedures were approved by the Animal Welfare and Ethical Review Body of the University of Bristol (UB/10/034) and the University of Exeter (2013/247), and the Marine Scotland Science named veterinarian. All procedures were deemed by the Home Office as being below the level of severity that would require licensing, although an appropriate licence was in place (PPL 30/2860).

2.3.2 Study species and holding conditions

For the behavioural experiment, 40 adult Atlantic salmon raised in captivity at the Marine Laboratory following collection as smolts from the wild (n = 32) or from a farm (Landcatch Ltd., Ormsary; n = 8) were used. The salmon (mean ± SE, wet mass: 3595 ± 152 g; total length: 678 ± 9 mm) were housed in outdoor tanks receiving ambient lighting conditions during the experimental period (May–June 2014).

For the physiological experiment, the 26 adult Atlantic salmon used had been fertilised and hatched at the Marine Laboratory from eggs and milt from broodstock at Aultbea. The salmon (mean ± SE, wet mass: 1320 ± 63 g; total length: 480 ± 5 mm) were housed indoors for the duration of the experiment (June–September 2014); lighting was provided by low-intensity green lamps.

All holding tanks (2–3 m diameter, 1 m water depth) received running seawater with a constant temperature of 10°C, and baseline dissolved oxygen concentrations of 8 mg/L. Fish were fed pellets at approximately 1–2% body weight per day. Acoustic conditions in the holding tanks were measured using a calibrated omnidirectional HTI-96-MIN hydrophone (frequency response = 2–30,000 Hz, sensitivity = -164.3 dB re 1V/µPa), and a Sony PCM-M10 24-bit recorder (44.1 kHz

sampling rate). Sound-pressure levels, analysed in MATLAB 2013a using the acoustics analysis package *PaPAM* (Nedelec et al. 2016a), were 130 and 138 dB re 1 µPa (10 s recording, 0–2000 Hz) for the behavioural and physiological holding tanks respectively.

2.3.3 Playback tracks and experimental design

Our primary aim was to test the impact of additional noise (playback of pile-driving) relative to responses of individuals raised in the same conditions but experiencing ambient-control conditions (playback of ambient-harbour sound). Any effect found is thus likely due to the additional noise and not from captivity. A collection of pile-driving and ambient-sound playback tracks, created from original recordings (Wale et al. 2013; Simpson et al. 2015; Radford et al. 2016b), were used for the experiments. Three ambient-sound recordings were made at each of three major UK harbours (Gravesend, Plymouth, Portsmouth), when no ships were present nearby. Pile-driving was recorded at 2–3 m depth in Swansea Bay, UK, 127 m from the sound source (a 1.2 m diameter monopole driven 25 m into the seabed within 6.5 m water depth) (Radford et al. 2016b); see Fig. 2.1 for a comparison of pressure waveforms from the original field recording and a recording of the experimental playback of a pile-driving strike. Three unique 5-min sub-sections from each recording were created using Audacity 2.0.3 (http://audacity.sourceforge.net), generating nine different ambient-sound playback tracks (e.g. A1), and three pile-driving tracks (e.g. P2). Each ambient-sound track was matched with one of the three pile-driving tracks to produce nine different pile-driving playback tracks, each containing underlying ambient-harbour sound (e.g. A1+P2).

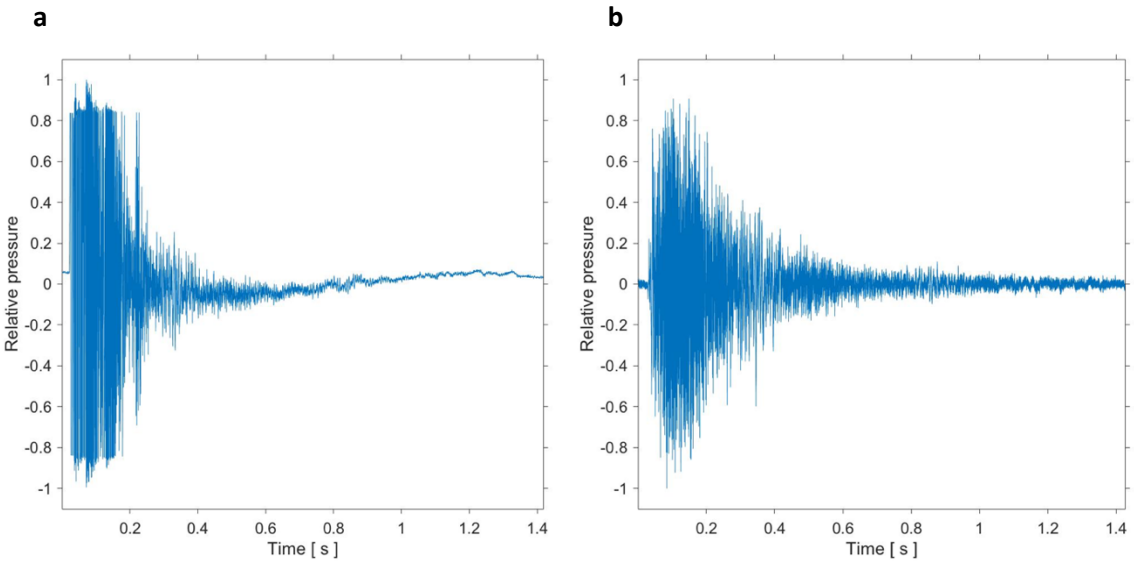

Figure 2.1. Relative sound-pressure waveform of a single pile-driving strike for (a) original and (b) loudspeaker playback in dumbbell testing tank (used for behavioural experiments; see below). Pile-driving strikes analysed in MATLAB 2017a using the acoustics analysis package *PAMGuide* (Merchant et al. 2015). Hamming evaluation window, 50% overlap, fft = 1024.

In both experiments, no sound treatment (ambient-sound or pile-driving noise) was tested consecutively for more than two days and the treatment order was randomised. In the behavioural experiment, the ambient-control treatment used only ambient-sound playback tracks (e.g. A1), whereas the pile-driving treatment used both pile-driving (e.g. A1+P1) and ambient-sound (e.g. A2) tracks. The total duration of each trial was 5 h. The first hour provided 'baseline' movement patterns during ambient-sound playback. Following this, fish in the pile-driving treatment were played a pile-driving track from one loudspeaker and an ambient-sound track from the other (see Fig. 2.2 for a schematic of the testing tank); playback track types were switched between ends each hour for a total of 4 h, with four pile-driving and four ambient-sound playback tracks being used per trial, excluding the 'baseline' measurement period. The end at which the initial pile-driving track was played was alternated between the 10 pile-driving trials to avoid any bias between the ends of the testing tank. The ambient-control trials followed the same procedure except a randomised combination of different ambient-sound tracks was played from both loudspeakers. Playback tracks were randomly assigned to each trial; the randomised combinations and use of different playback tracks within each trial minimised pseudoreplication. Fish origin (wild or farmed) was counterbalanced between the two treatments to prevent bias of origin influencing behavioural

responses. In the physiology experiment, a single playback track was used per trial, with the track randomly assigned to individual fish.

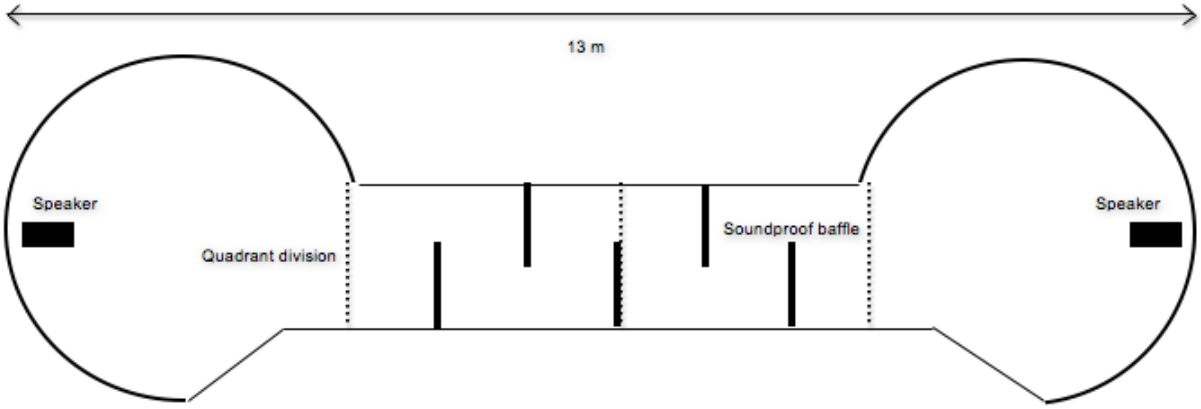

Figure 2.2. Schematic of the dumbbell tank used for the behavioural experiment. Shown are the approximate positions of the soundproof baffles, quadrant divisions (dashed lines) and speakers.

2.3.4 Behavioural experiment

Two Dyna-Empire J9 sound projectors (Dyna-Empire Inc. New York, USA), each connected to a 40 W mini amplifier (LP-2020A + Lepai Tripath class-T Hi-Fi audio mini amplifier), were positioned at either end of a 13 m dumbbell-shaped tank (Fig. 2.2). Five soundproof baffles were used to attenuate the sound from the playback tracks and create a reduction in sound level between the two ends of the tank. Prior to each trial, two Atlantic salmon were transferred into the tank and given an overnight acclimation period to recover from the stress of transfer and to explore the experimental arena. Fish were tested in pairs to reduce stress associated with social isolation (Doyon et al. 2005). Trials were filmed using three overhead CCTV video cameras for subsequent analysis. Twenty pairs of Atlantic salmon were used in an independent-measures design. The fish were terminated using a Home Office Schedule 1 method at the end of the trial, and subsequently weighed and measured. There was no significant difference in the mass (two sample t-test: $t = 0.11$, $df = 38$, $p = 0.915$) or total length ($t = 0.04$, $df = 38$, $p = 0.968$) of fish randomly assigned to the two treatments.

To assess short-term behavioural responses to pile-driving noise, the presence/absence of a startle response (rapid directional change in swimming trajectory between consecutive frames; Simpson et al. 2015) was determined for the pair of salmon at the onset of pile-driving noise, and equivalent time in the ambient-control trials, following the 'baseline' measurement period. Acute

stressors have been shown to cause increases in swimming speed as an avoidance response (Herbert and Steffensen 2005). So, the swimming speed (body lengths/s) of both fish in the 10 s following the onset of pile-driving playback and during the equivalent time period in the ambient-control trials was determined. All videos were analysed by H.R.H. who was 'blind' to the acoustic treatment.

The quarter of the tank the fish were in was recorded every minute for the duration of the trial; combined fish positions were used to calculate a percentage of the total time fish spent in either end section ('near', 'far'), with the end playing pile-driving classified as 'near'. For the ambient-control trials, the accompanying pile-driving trial in the randomised block of two was used to define the near and far ends. The percentage difference in time spent at the near end between the 'baseline' and the first exposure hour was also assessed.

2.3.5 Physiological experiment

Oxygen-consumption rate of individual fish, relative to body mass, was determined over a 2 h period using a static respirometer (303 L) submerged within an annular tank (10 m diameter, 1 m water depth). A Lubell LL916C underwater loudspeaker (Lubell Labs Inc. Columbus, Ohio, USA), connected to a 40 W mini amplifier (LP-2020A + Lepai Tripath class-T Hi-Fi audio mini amplifier), was positioned 2 m away at a depth of 0.5 m. Twenty-six fish were used in an independent-measures design, exposed to either ambient-sound or pile-driving playback. At the end of a trial, the fish was terminated using a Home Office Schedule 1 method and subsequently weighed and measured. There was no significant difference in the mass (two sample t-test: $t = -0.16$, $df = 21$, $p = 0.877$) or total length ($t = -0.99$, $df = 21$, $p = 0.334$) of fish randomly assigned to the two treatment groups.

Prior to each trial, an individual salmon was transferred into the chamber and given an overnight acclimation period. The chamber was then sealed and the playback track started. An oxygen concentration cut-off value of 6 mg/L was imposed as the point at which a trial would be stopped early to avoid hypoxia; this cut-off value was never reached. Dissolved oxygen concentrations and water temperature were recorded every 150 s using an oxygen probe with inbuilt thermometer (Handheld dissolved oxygen meter; HI-9146N, Hanna Instruments Ltd). A closed-circuit recirculation loop containing the oxygen probe avoided localised depletions of dissolved oxygen and increased the ability of the static respirometer to detect differences between individuals (Rodgers et al. 2016). A high-definition video camera was used to characterise any movement during the trial, from which a total percentage activity was calculated.

Mass-corrected oxygen-consumption rates were calculated using the initial and final dissolved oxygen concentrations of each trial. Consumption rate was calculated by converting the change in oxygen (ppm) over the duration of the trial into micromoles of oxygen used per gram of fish per hour (µmoles O_2 g^{-1} h^{-1}). A seawater salinity of 35 (g/L), and the known average temperature for each trial, was used to calculate water density. A specific density per trial in g/cm^3 was used to determine accurate concentrations of dissolved oxygen. These were applied to each trial to report the total dissolved oxygen consumed relative to the mass of each fish per hour. Oxygen-consumption rate for each fish was plotted against its corresponding activity assessment. The gradient of the slope was multiplied with the activity of the trial. The resultant values, representing an estimated oxygen-consumption rate when the fish was active, were subtracted from the original consumption rates. This gave an oxygen-consumption rate that factored in variances in activity levels between trials. Three trials were dropped from the analysis due to a lack of fish-activity data.

2.3.6 Acoustic analysis

Sound-pressure levels in experimental tanks were measured using a calibrated omnidirectional hydrophone and digital recorder (details as above). Particle-motion levels were measured using a calibrated triaxial accelerometer (M20L, sensitivity 0–3 kHz, Geospectrum Technologies) and digital 4-track recorder (Boss BR-800, 44.1 kHz sampling rate, Roland Corporation, Los Angeles, CA). All recording levels used were calibrated with pure sine waves from a function generator with a known voltage recorded on an oscilloscope. Pressure and particle-motion analysis was conducted in MATLAB 2013a using the acoustics analysis package *PaPAM* (Nedelec et al. 2016a). Sound propagation along the dumbbell tank was assessed using illustrative acoustic waveforms of individual pile-driving strikes recorded at various distances from the loudspeaker (Fig. 2.3a, 2.3b). See Table 2.1 for mean sound-pressure levels (zero-peak) ($SPL_{0\text{-}p}$), mean sound-exposure levels (single strike) (SEL_{ss}), and cumulative sound-exposure levels (SEL_{cum}) over the 4 h pile-driving duration at increasing distances from the sound source in the dumbbell tank. Ambient-sound playback and background noise were 128 and 117 dB re 1µPa (10 s recording, 0.1 m from sound source; 0–2000 Hz) respectively. The particle-motion waveform (including cumulative power) of a single pile-driving strike was calculated (Fig. 2.4a, 2.5a). Spectrograms and power spectral densities were calculated for representative examples of pile-driving and ambient-sound playback tracks in both experimental setups (Fig. 2.4b–2.4f, 2.5b–2.5f). In the physiology chamber, sound-pressure levels were 126 (ambient-sound playback) and 120 (background) dB re 1 µPa (10 s recording; 0–2000 Hz). See Table 2.1 for mean $SPL_{0\text{-}p}$, mean SEL_{ss} and SEL_{cum} over the 2 h pile-driving period.

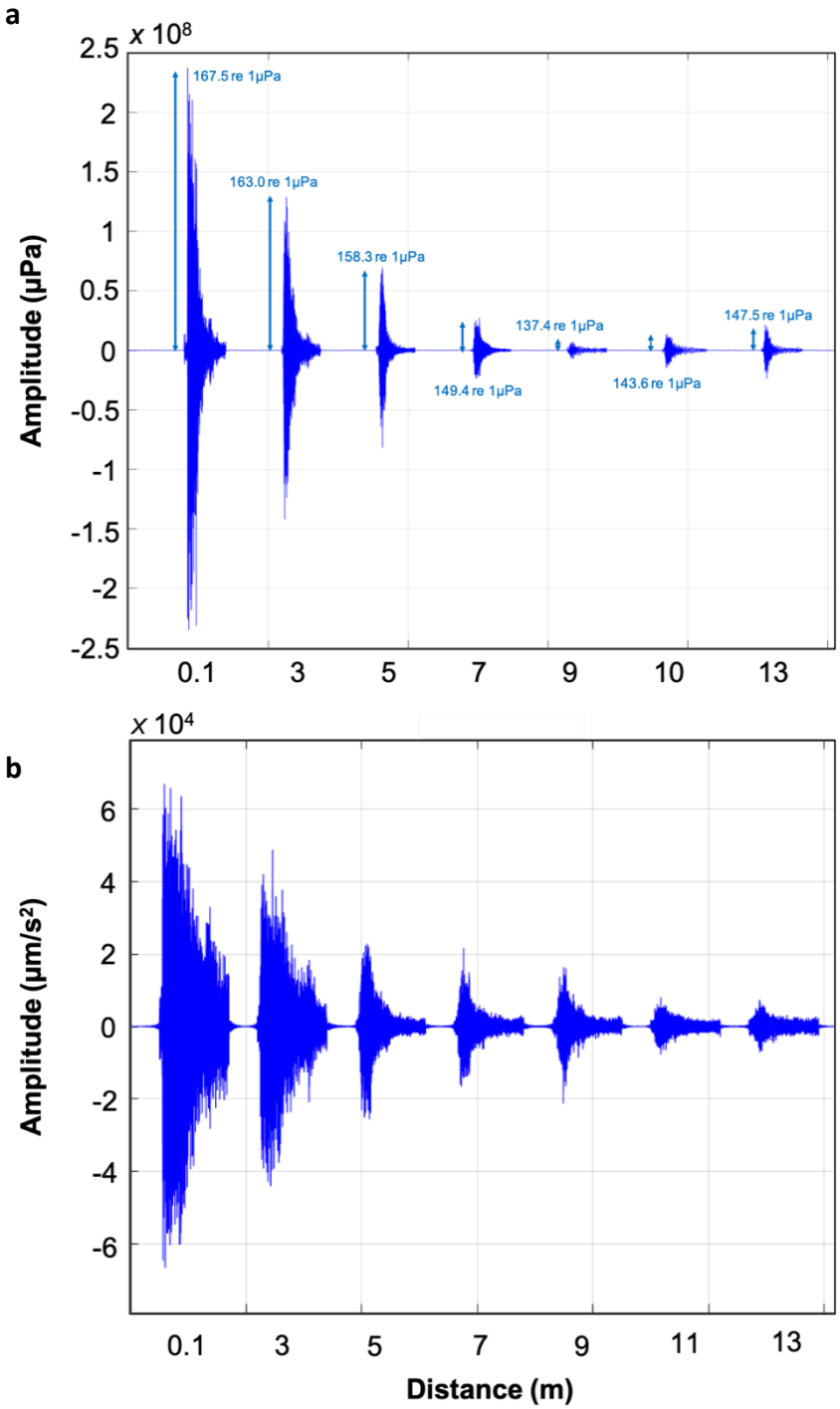

Figure 2.3. Sound propagation of acoustic waveforms along the dumbbell tank. (a) Representative sound-pressure waveforms of individual pile-driving strikes recorded at increasing distance from the loudspeaker. Blue arrows indicate the zero–peak sound-pressure level of single strikes. (b) Equivalent representative particle-motion waveforms using the X axis of the triaxial accelerometer. Hamming evaluation window, 50% overlap, fft = 1024. Analysis performed in MATLAB 2013a using the acoustic analysis package *PaPAM* (Nedelec et al. 2016a).

Table 2.1. Pile-driving sound-pressure and particle-motion analysis for the behavioural and physiological experiments. Mean sound-exposure levels (single-strike), and mean sound-pressure level (zero–peak) both calculated over 8–10 individual pile-driving strikes in the respirometer and at increasing distances along the dumbbell tank. The minimum and maximum values are provided for the 8–10 strikes analysed. SEL_{cum} was calculated, as per Bolle *et al.* (2012), for the total duration of each trial using the mean SEL_{ss}.

EXPERIMENT	Sound metrics	Units	Distance from source (m)								No. of strikes
			0.1	2	3	5 (**X-only**)	7	9	10 (**11**)	13	
BEHAVIOURAL	Mean $SPL_{0\text{-}p}$	dB re 1 µPa	168.0		163.9	157.7	149.0	136.4	143.9	146.6	10
	Range		167.4–169.6		162.8–165.4	154.1–158.8	147.4–150.4	135.0–137.8	141.7–145.3	144.5–147.9	
	Mean SEL_{ss}	dB re 1 µPa² s	152.8		146.0	139.1	130.8	122.7	126.6	129.2	
	Range		152.0–154.8		145.4–147.4	137.0–140.0	129.5–132.4	121.2–124.0	125.2–127.6	127.7–131.0	
	SEL_{cum}		192.9		186.1	179.2	170.9	162.8	166.7	169.3	10,286
	Mean SEL_{ss}	dB re (1µm/s)² s	88.10		81.83	**71.60**	73.53	74.45	**69.38**	68.79	8–10
	Range		87.83–88.57		81.40–82.11	**71.24–72.18**	72.93–74.37	73.67–74.95	**68.54–69.86**	67.76–70.49	
	SEL_{cum}		128.22		121.95	**111.72**	113.65	114.57	**109.50**	108.91	10,286
PHYSIOLOGICAL	Mean $SPL_{0\text{-}p}$	dB re 1 µPa		173.0							10
	Range			172.5–173.5							
	Mean SEL_{ss}	dB re 1 µPa² s		157.3							
	Range			156.4–157.9							
	SEL_{cum}			194.4							5,143
	Mean SEL_{ss}	dB re (1µm/s)² s		94.19							9
	Range			92.91–95.33							
	SEL_{cum}			131.30							5,143

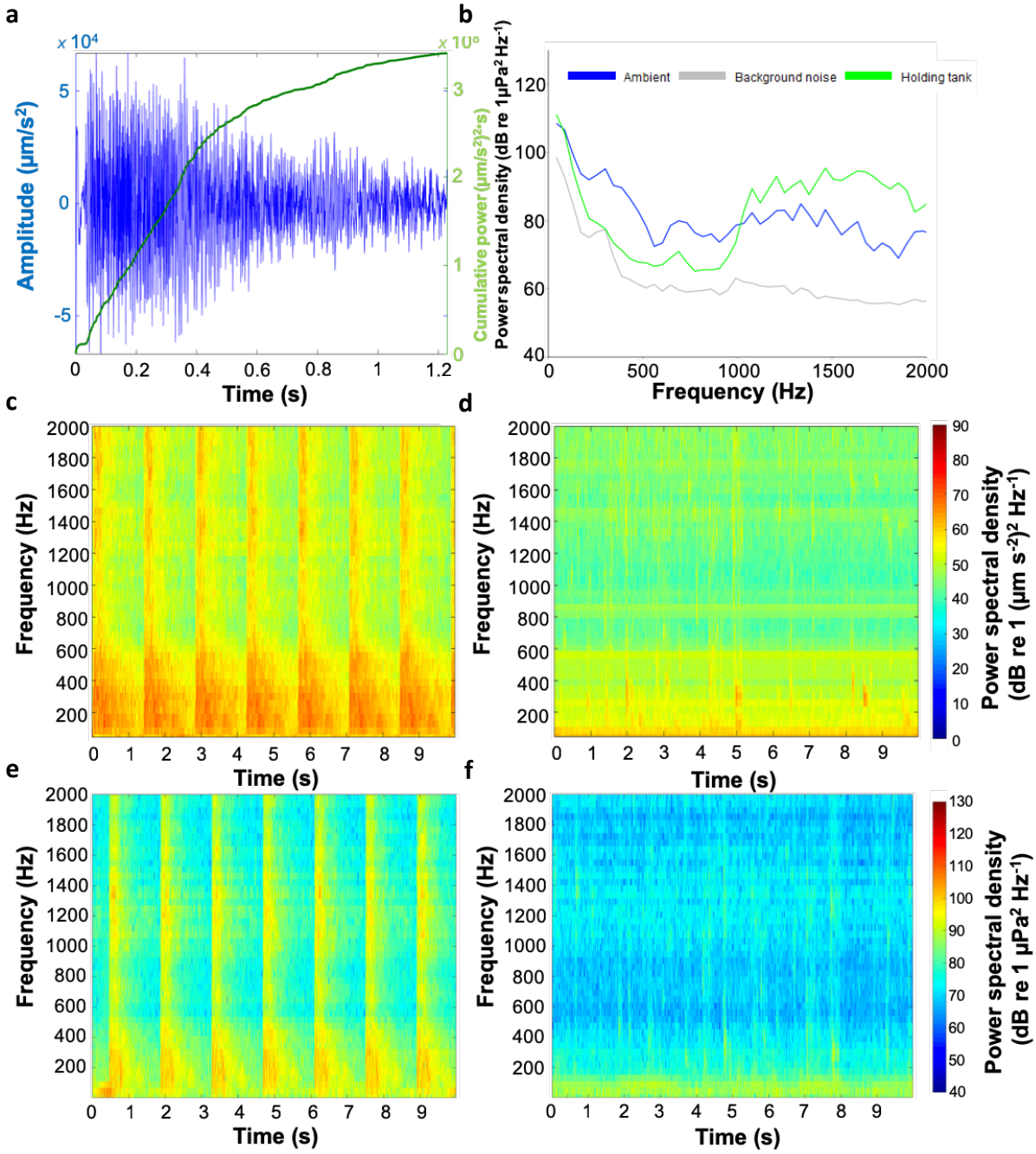

Figure 2.4. Behavioural experiment sound analysis. (a) Representative example of a particle-motion waveform of a single piling-driving strike, including the cumulative power curve, for the X axis of a triaxial accelerometer. (b) Power spectral density of 10 s ambient-sound playback, holding tank and background noise of the dumbbell tank. (c–d) Triaxial particle motion of 10 s of pile-driving and ambient-sound playback, respectively. (e–f) Acoustic-pressure spectrogram of 10 s of pile-driving and ambient-sound playback, respectively. Hamming evaluation window, fft = 1024, 50% window overlap, Bandpass filtered: 0–2 kHz, 0.1 m from loudspeaker. Analysis performed in MATLAB 2013a using the acoustic analysis package *PaPAM* (Nedelec et al. 2016a).

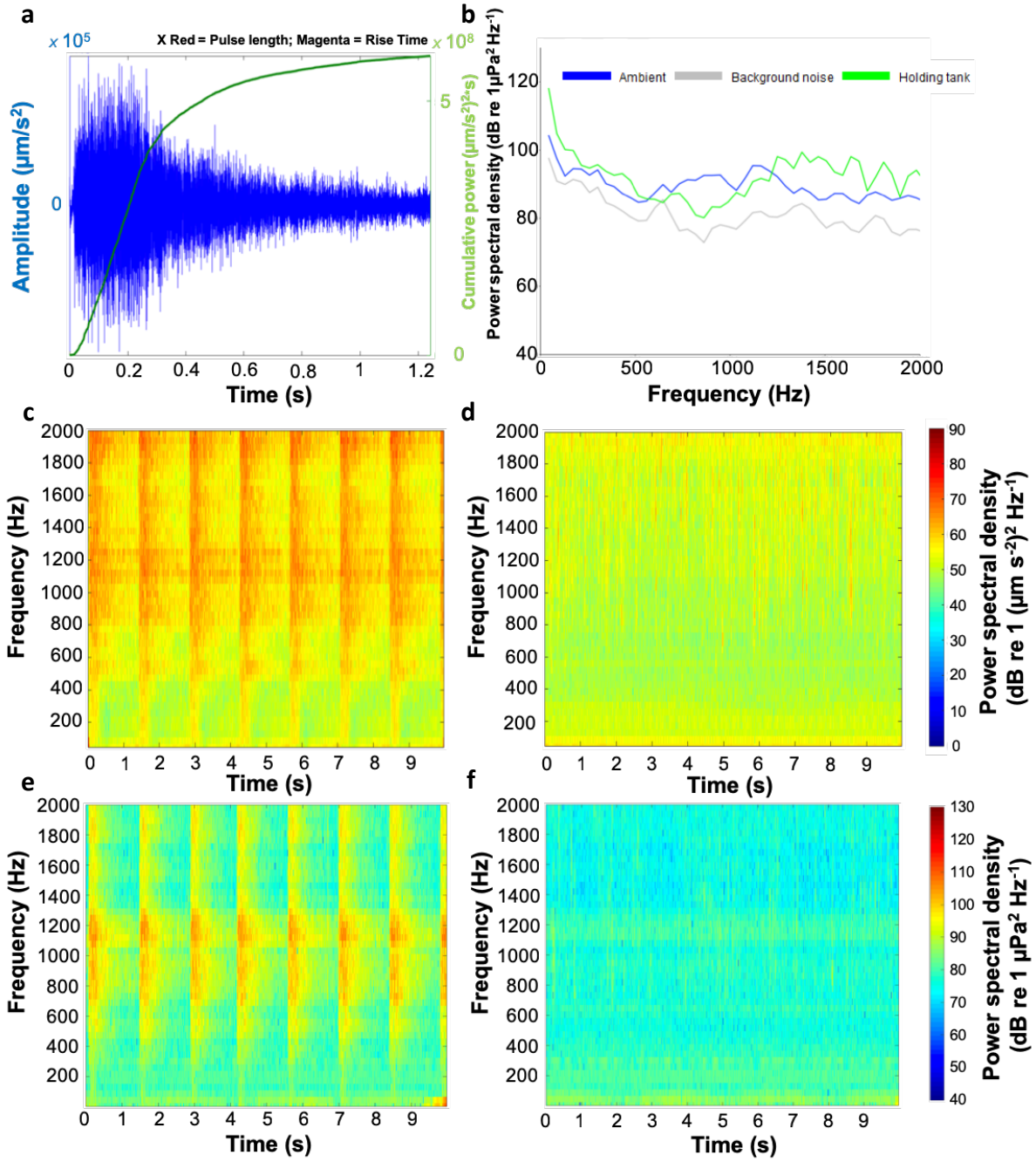

Figure 2.5. Physiological experiment sound analysis. (a) Representative example of a particle-motion waveform, using the X axis of a triaxial accelerometer, of a single pile-driving strike including the cumulative power curve. (b) Power spectral density of 10 s ambient-sound playback, holding tank and background noise of the annular tank. (c–d) Triaxial particle motion of 10 s of pile-driving and ambient-sound playback, respectively. (e–f) Acoustic-pressure spectrogram of 10 s of pile-driving and ambient-sound playback, respectively. Hamming evaluation window, fft = 1024, 10 s recording, 50% window overlap, Bandpass filtered: 0–2 kHz. Analysis performed in MATLAB 2013a using the acoustic analysis package *PaPAM* (Nedelec et al. 2016a).

2.3.7 Statistical analysis

All statistical analyses were carried out in R (Version 3.2.2). Normality of residuals and homogeneity of variances were visually assessed for each dataset and the appropriate test (two-sample t-test or Mann-Whitney U test) used.

2.4 RESULTS

2.4.1 Behavioural experiment

No startle response was observed for any fish at the onset of either ambient-sound playback or pile-driving playback. There was no significant difference in swimming speed (body lengths/s) in the 10 s immediately following the onset of pile-driving noise compared to the equivalent period in the ambient-control trials (Mann Whitney U test: $W = 66$, $n_{control} = 9$, $n_{pile\text{-}driving} = 10$, $p = 0.09$; Fig. 2.6a).

Sound treatment had a significant influence on the amount of time fish spent at either end of the dumbbell tank during the 4 h experimental-exposure period: fish spent more time in both the near-end (Mann-Whitney U test: $W = 23.5$, $n_{control} = 10$, $n_{pile\text{-}driving} = 10$, $p = 0.049$; Fig. 2.6b) and the far-end ($W = 18$, $n_{control} = 10$, $n_{pile\text{-}driving} = 10$, $p = 0.017$; Fig. 2.6c) when experiencing ambient-sound playback compared to pile-driving playback. In baseline conditions, there was no significant treatment-based difference in the percentage time spent in the near-end ($W = 53.5$, $n_{control} = 10$, $n_{pile\text{-}driving} = 10$, $p = 0.82$; Fig. 2.6d) or far-end of the tank ($W = 31$, $n_{control} = 10$, $n_{pile\text{-}driving} = 10$, $p = 0.162$; Fig. 2.6e). After controlling for fish location during the 'baseline' period, by examining the change in position once playback started, there was no such effect of sound treatment during the exposure period. There was no significant treatment-based difference in time spent in the near-end ($W = 67.5$, $n_{control} = 10$, $n_{pile\text{-}driving} = 10$, $p = 0.198$; Fig. 2.6f) or the far-end ($W = 51.5$, $n_{control} = 10$, $n_{pile\text{-}driving} = 10$, $p = 0.94$; Fig. 2.6g) between the 'baseline' hour and the first hour of the experimental sound treatment.

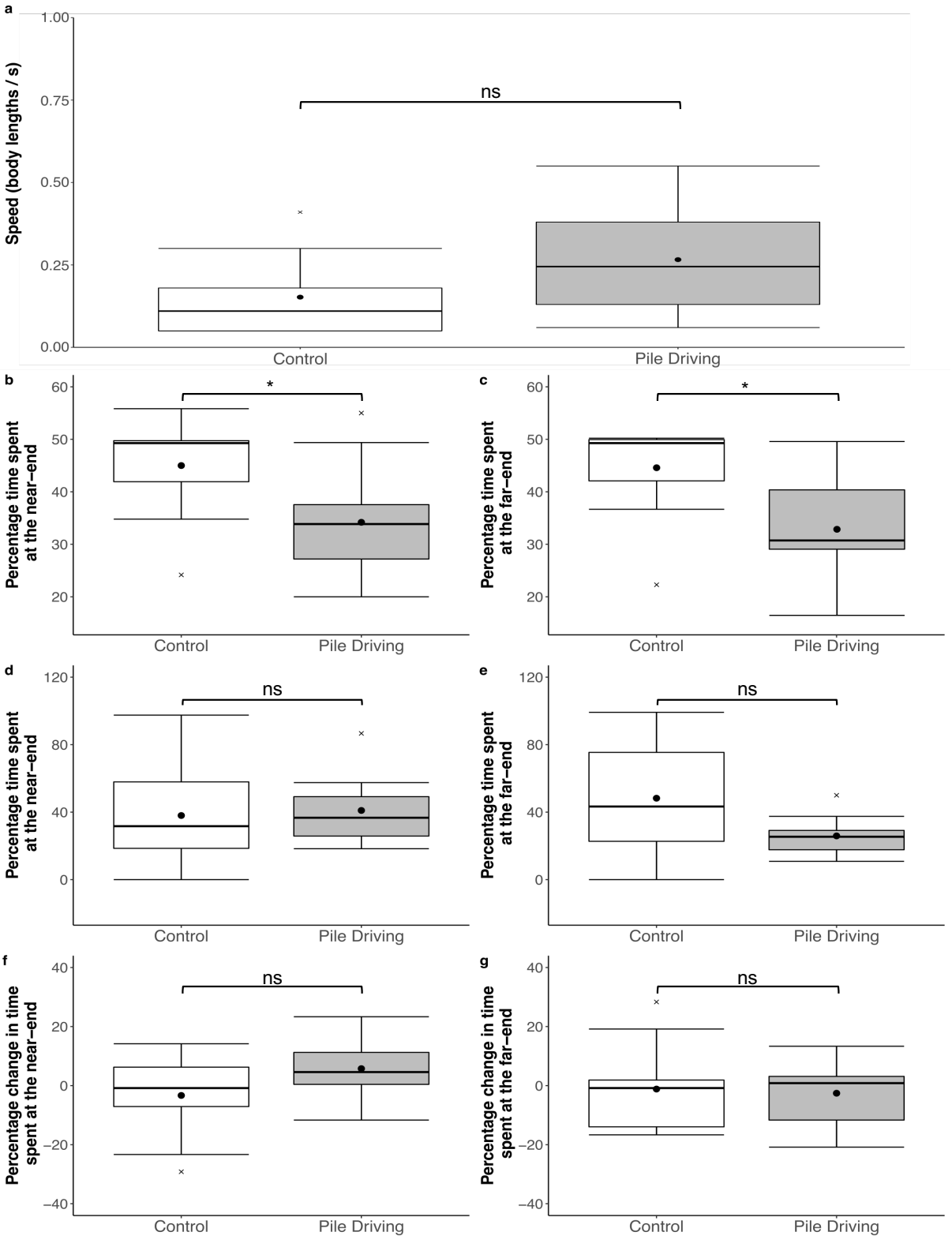

Figure 2.6. Atlantic salmon behavioural responses to pile-driving playback. (a) Boxplot for the mean speed (body lengths/s) during the 10 s following the onset of pile-driving, and equivalent period in the ambient-control trials ($n_{control}$ = 9, $n_{pile\text{-}driving}$ = 10). Boxplots for the percentage time Atlantic salmon spent at the (b) near-end (section with loudspeaker playing pile-driving noise or its

equivalent in ambient-control trials; $n_{control} = 10$, $n_{pile\text{-}driving} = 10$) and (c) far-end (section furthest from the loudspeaker playing pile-driving noise or its equivalent in ambient-control trials; $n_{control} = 10$, $n_{pile\text{-}driving} = 10$) over the 4 h exposure period. Percentage time spent at the (d) near-end ($n_{control} = 10$, $n_{pile\text{-}driving} = 10$), and (e) far-end ($n_{control} = 10$, $n_{pile\text{-}driving} = 10$) during the baseline period. Percentage change between the 'baseline' and first exposure hours in the proportion of time Atlantic salmon spent at the (f) near-end ($n_{control} = 10$, $n_{pile\text{-}driving} = 10$) and (g) far-end ($n_{control} = 10$, $n_{pile\text{-}driving} = 10$). For each boxplot, the central line represents the median and the top and bottom box lines represent the upper (75th) and lower (25^{th}) percentiles, respectively. Upper and lower whiskers are 1.5*IQR, crosses represent outliers, and the black circles the means. ns = non-significant; * $p < 0.05$.

2.4.2 Physiological experiment

Activity level was shown to be a strong predictor of oxygen-consumption rate for individual fish (Regression analysis: $R^2 = 0.55$, $F_{1,21} = 25.5$, $p < 0.005$; Fig. 2.7a). Although, there was no significant difference in mean activity level between treatment groups (Mann-Whitney U test: $W = 59$, $n_{control} = 11$, $n_{pile\text{-}driving} = 12$, $p = 0.695$). Fish exposed to playback of pile-driving noise did not differ significantly in their oxygen-consumption rate compared to fish exposed to ambient-sound playback (two-sample t-test: $t = 0.62$, $df = 21$, $p = 0.539$; Fig. 2.7b).

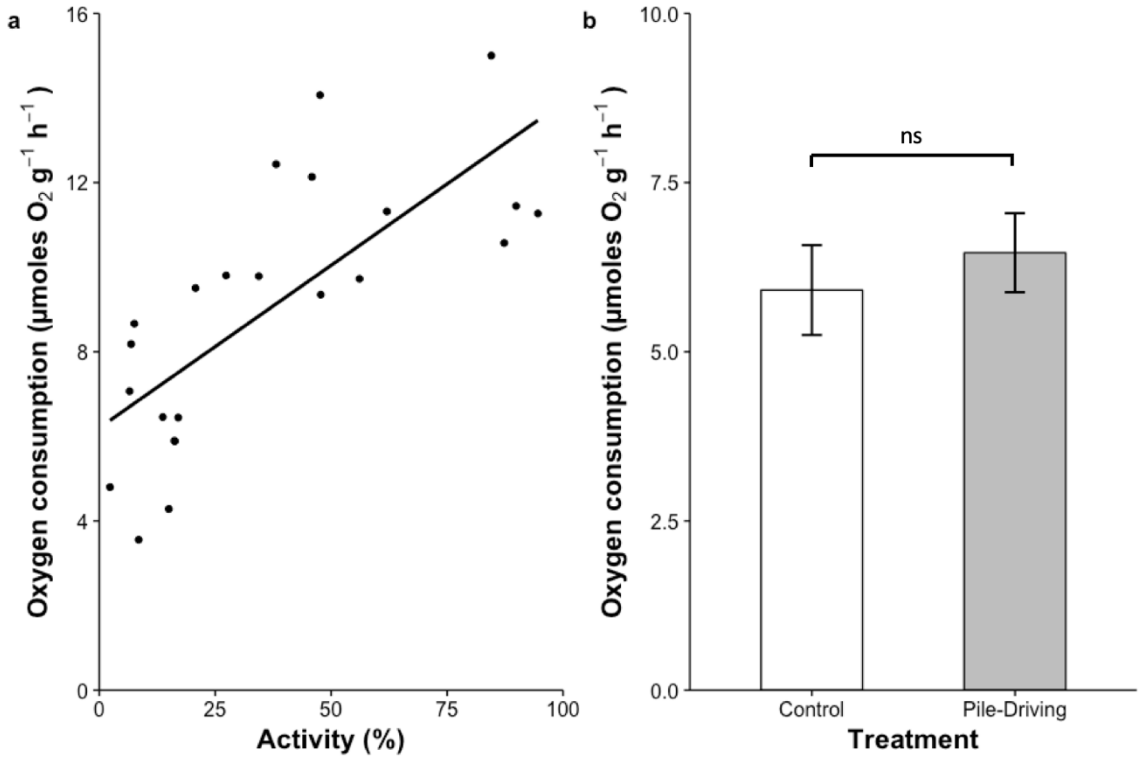

Figure 2.7. Atlantic salmon physiological response to pile-driving playback. (a) Correlation between oxygen-consumption rate relative to body mass and recorded activity level (% time spent moving). Activity level was shown to be a strong predictor of oxygen-consumption rate (Regression analysis: $R^2 = 0.55$, $F_{1,21} = 25.5$, $p < 0.005$). (b) Mean ± SE activity-corrected oxygen-consumption rate relative to body mass of fish exposed to playback of either pile-driving noise or ambient sound. ns = non-significant, $n_{control} = 11$, $n_{pile\text{-}driving} = 12$.

2.5 DISCUSSION

Pile-driving playback had no significant effect on the measured behavioural responses of Atlantic salmon. No startle response was evident in any of the fish at the onset of pile-driving and there was no increase in swimming speed relative to the ambient controls. Salmon in the ambient-control treatment spent significantly more time at either end of the dumbbell tank compared to those in the pile-driving treatment. However, the identified treatment difference was already becoming apparent in the baseline period prior to the start of the sound treatment for the percentage time fish spent at the far-end, although not significant. Thus, there was no treatment-based difference in the proportion of time spent at either the near-end or far-end between the 'baseline' and the first exposure hour. This strongly suggests that additional noise of pile-driving is not the cause of the

observed differences in behaviour. This identified artefact highlights the possible bias that can result despite random allocation of fish to treatments, exacerbated by small sample sizes, and stresses the importance of identifying baseline behaviour for use in change-based analysis. The lack of discernible behavioural responses observed here are similar to the findings of previous work investigating behavioural responses of a closely related species (*Salmo trutta*) to real pile-driving noise (Nedwell et al. 2003). Additionally, in our study, Atlantic salmon did not show a difference in oxygen-consumption rate when exposed to pile-driving noise compared to the ambient-control treatment.

One possible explanation for the general lack of responses to playback of pile-driving noise is that the salmon may have detected the pile-driving stimulus but did not perceive the acoustic disturbance as a stressor; oxygen-consumption rate may be used as an indicator of stress due to its relationship with stress hormones (Morgan and Iwama 1996; Barton 2002; Nadler et al. 2016). This explanation seems unlikely as pile-driving noise has been shown to affect physiology in other fish species that have a greater spectral hearing range, and therefore likelihood of detecting such a stimulus (Lovell 2003; Bruintjes et al. 2016a,b; Radford et al. 2016b). Alternatively, the lack of response may centre on the hearing ability of Atlantic salmon. Compared to other teleost fish, including Atlantic cod (*Gadus morhua*) and herring (*Clupea harengus*), Atlantic salmon are relatively sound insensitive and lack specialist hearing mechanisms (Chapman and Hawkins 1973; Hawkins and Johnstone 1978). The absence of such mechanisms reduces the sensitivity and spectral range of hearing, resulting in a poorer ability to distinguish specific acoustic cues from background noise (Hawkins and Johnstone 1978; Popper and Fay 1993; Kenyon et al. 1998; Radford et al. 2012). Atlantic salmon have shown avoidance to infrasound (<20 Hz) but not when tested at higher frequencies (e.g. 150 Hz) (Knudsen et al. 1992; Bui et al. 2013), despite showing a lowered heart rate in response to pure tones below 380 Hz (Hawkins and Johnstone 1978). Testing avoidance responses at a range of frequency bandwidths and sound levels, measuring both particle motion and sound pressure, would develop our understanding of the link between behaviour and hearing abilities of fishes.

The present study used adult Atlantic salmon, with all cohorts of fish kept in captivity since hatching or brought in as smolt (wild-caught, except for eight farm-sourced fish). There is increasing evidence that farmed/hatchery-reared salmon have otoliths (ear bones) formed from vaterite, which is calcium carbonate in an alternative structure than the normally present aragonite found in wild conspecifics (Sweeting et al. 2004; Reimer et al. 2016). The underlying cause is still largely unknown, but research suggests that vateritic replacement can affect otolith functioning, including hearing

ability (Oxman et al. 2007; Reimer et al. 2016). Otolith morphology was not examined in this study so it is not possible to assess whether the fish used were affected by vaterite replacement; however, responses were qualitatively consistent among fish from different origins across all behavioural metrics.

The acoustic history of the animals may also have contributed to the lack of behavioural and physiological responses observed. Aquarium facilities are inherently noisy places, with aerators, pumps and filtration systems significantly raising noise levels (Wysocki et al. 2007). Previous studies on a range of species with varying hearing abilities have found temporary changes in hearing thresholds can result from exposure to increased levels of continuous noise (Smith et al. 2004; Wysocki and Ladich 2005; Halvorsen et al. 2009). There are currently no data on whether noise from hatchery systems can cause hearing threshold shifts in captive Atlantic salmon. Further research is needed to identify how hearing sensitivities of such fish compare to wild conspecifics at a similar developmental stage. This will aid in determining the suitability of captive fish as a model for wild Atlantic salmon when testing for impacts of acoustic disturbance.

In this study, we fully characterised both elements of the sound field (pressure and particle motion) for each experiment. This provided unique data on Atlantic salmon responses to defined particle-motion values. Particle-motion data represent a vital component in developing a comprehensive understanding of how anthropogenic noise affects aquatic organisms, especially fish and invertebrates (Nedelec et al. 2016a; Hawkins and Popper 2016); Atlantic salmon are primarily sensitive to particle motion and not acoustic pressure (Hawkins and Johnstone 1978). Early work on the impacts of anthropogenic noise on fish and invertebrates tended to include only sound-pressure recordings, if any measure of the sound field and acoustic stimulus was provided at all. Whilst there is a much-needed increase in the characterisation and reporting of particle-motion levels in such studies, especially those conducted in open-water conditions (Nedelec et al. 2016b; Neo et al. 2016; Simpson et al. 2016a; Neo et al. 2018), recordings from a range of noise sources, including pile-driving, under varying environmental conditions are still required (Merchant et al. 2015; Nedelec et al. 2016a).

Biological response measures from free-swimming fish in unconstrained environments provide the most ecological validity in terms of assessing movement responses to noise (Hawkins et al. 2014; Roberts et al. 2016; Slabbekoorn 2016). However, using fish in tank-based experiments provides the opportunity to minimise confounding variables usually present in fieldwork, and to

collect accurate and detailed response data (Halvorsen et al. 2012; Simpson et al. 2015). The caveats described here highlight the need to explore responses of recently caught wild fish in a similar experimental setup used in the current study, to help indicate the usefulness of captive salmon as a model for wild conspecifics when investigating noise pollution. To complement the present study, further experiments are needed using exposures to real noise sources in open soundscapes (see Debusschere *et al.* 2016 as an example), and also testing wild-caught salmon in natural conditions. By using a range of methodologies across different testing systems (field/laboratory), a holistic understanding can be developed regarding organismal responses to underwater noise pollution (Slabbekoorn 2016). The use of marine renewable energy devices (e.g. offshore wind turbines) represents an important avenue for development and expansion in order to aid the transition to a net-zero carbon economy (Pye et al. 2017). It is increasingly likely, therefore, that organisms will be exposed to anthropogenic noise during construction and operation of such devices in the future. Thus, a fuller understanding of organismal responses to anthropogenic noise is vital if we are to mitigate any potential impacts. This study provides an important first step for developing a comprehensive understanding of responses to impact pile-driving.

Chapter 3 – Condition-dependent responses of *Chromis viridis* to motorboat noise

Contributions to the work

H.R.H., T.A.C.G. (University of Exeter PhD student; acted as field assistant) and supervisors S.D.S. and A.N.R. conceived the research; H.R.H., T.A.C.G., M.I.M. (Professor of Coral Reef Ecology at James Cook University; provided valuable local advice during fieldwork), S.D.S. and A.N.R. designed the experiments; H.R.H., T.A.C.G. and K.W. (US-based School for International Training Student; acted as field assistant) conducted the fieldwork. H.R.H extracted and analysed the data, and interpreted the results, advised by S.D.S. and A.N.R.; H.R.H. wrote the chapter, with comments provided by S.D.S. and A.N.R.

Elements of this chapter are being prepared for publication as:
Harding HR, Gordon TAC, Wong K, McCormick MI, Simpson SD, Radford AN. Condition-dependent responses of *Chromis viridis* to motorboat noise. *Biol Lett*.

3.1 ABSTRACT

Anthropogenic noise is a pollutant of global concern, and has been shown to have a wide range of effects on multiple taxa. Yet, the majority of these studies have only considered the effects of noise using overall population means, ignoring potential intraspecific variation in responses. The impact of anthropogenic stressors can vary due to intrinsic characteristics (e.g. body size, sex, condition) and extrinsic factors (e.g. environmental context, prior experience), but experimental tests on fish using real noise sources are rare. Here, we assessed how differences in body condition and habitat quality influence the responses of *Chromis viridis* (a common coral reef fish) to real motorboat noise. Opercular beat rate (a measure of ventilation rate and increasingly used to consider the effect of anthropogenic stressors on fish) was unaffected by exposure to motorboat noise, with no differences in response shown by fish in relatively good and poor body condition. However, compared to better-condition conspecifics, poorer-condition individuals only startled to a looming stimulus (mimicking the approach of a predator) when it was significantly closer to them during exposure to motorboat noise; startle responses were equivalent for relatively good and poor condition fish under ambient conditions. In a multi-site experiment, habitat quality did not significantly affect responses to motorboat noise. These results suggest that we should be considering the variation within a population in order to determine accurately the effects of anthropogenic noise on animals. Failure to do so, risks misrepresenting potential effects with consequences on management and mitigation of this pervasive pollutant.

3.2 INTRODUCTION

Anthropogenic noise permeates multiple biomes and has become a pollutant of global concern, now included in environmental legislation around the world (Hildebrand 2005; Slabbekoorn et al. 2010; Buxton et al. 2017). Such noise has been shown to have a wide range of effects on many different taxa (for recent reviews, see: Shannon et al. 2015; Kunc et al. 2016). Recreational motorboats represent a large proportion of marine traffic in coastal regions (Whitfield and Becker 2014). Motorboat noise has been shown to affect the behaviour (e.g. orientation, communication, foraging, anti-predator responses) and physiology (e.g. opercular beat rate, metabolic rate, heart rate) of fish, and to have direct consequences for fitness (Holles et al. 2013; Whitfield and Becker 2014; Simpson et al. 2016a). Recreational boat use on the Great Barrier Reef (GBR) is forecasted to increase dramatically over the next few decades (Great Barrier Reef Marine Park Authority 2014), likely bringing marine fauna into contact with acoustic disturbances more frequently. It is therefore

paramount to have a comprehensive understanding of the potential impacts of anthropogenic noise on fishes to underpin mitigation and conservation efforts.

To date, the majority of studies testing for impacts of anthropogenic noise have predominantly considered only whether noise has an effect (Chapter 1); using trait means to assess responses from a cohort (Radford et al. 2016a). Intraspecific variation, such as from intrinsic characteristics (e.g. size, sex, body condition) and extrinsic factors (e.g. prior experience, environmental context), can affect animal responses to anthropogenic stressors (Harding et al. 2019). Failing to consider intraspecific variation risks misinterpreting the full impacts of noise (Radford et al. 2016a; Harding et al. 2019), and may prevent effective mitigation strategies being developed that protect all individuals in a population. There is a growing body of work showing the influence of intraspecific variation in responses to noise (see Table A.1, Appendix A for examples) but more experimental tests are required.

Body condition can vary greatly between individuals within a population, and can be affected by external factors (e.g. parasitism, food availability, environmental conditions) and internal characteristics (e.g. reproductive state, age) (Brosset et al. 2015). Conditional state can affect decision-making in animals, with malnourished individuals likely to display more risk-prone behaviours in order to continue foraging despite the increased threat of predation (Biro and Booth 2009; Sih and Del Giudice 2012). Body condition has been shown to affect responses to natural and anthropogenic stressors (Mubiana et al. 2006; Dissanayake et al. 2008; Reid and Purcell 2011). For instance, herbivorous mountain pine beetles (*Dendroctonus ponderosae*) in 'average' to 'poor' condition had less than 8% survival following exposure to host-plant monoterpenes, whereas there was no effect of monoterpene concentration on the survivorship of 'good' condition beetles (Reid and Purcell 2011). To our knowledge, however, there is only one study demonstrating condition-dependent responses to anthropogenic noise: laboratory-based experiments found that European eels (*Anguilla anguilla*) in poorer physiological condition were more susceptible than those in better condition to noise playbacks (Purser et al. 2016). To date, condition-dependent responses to noise have not been examined *in situ* using real noise sources.

Habitat quality within aquatic environments is heterogenous (Griffen and Norelli 2015). Differences in the quality of habitat within an environment can result in intraspecific variation in individual behaviour and physiology (Schrandt et al. 2011; Schrandt and Lema 2011; McCormick 2012; Boström-Einarsson et al. 2018). One such physiological difference may be that individuals in

worse habitat have a poorer body condition than those from better habitat (Oliva-Paterna et al. 2003). This difference could be the result of better-condition individuals outcompeting poorer-quality conspecifics for access to the better-quality habitat or be because individuals in worse habitat have access to fewer resources or are under other increased pressures which leads to a reduction in body condition. A reduced body condition may subsequently affect responses to additional challenges (such as noise) as discussed above. It may also be inherently more stressful to inhabit areas of poorer quality; individuals from poorer-quality habitat can show an increase in baseline stress hormones (Homan et al. 2003; Martínez-Mota et al. 2007; King et al. 2016). Elevated baseline stress or previous experience of a stressful event can affect responses to further stressful episodes (Barton et al. 2005; Cook et al. 2012; Mills et al. 2015). Multiple stressors can result in responses that are, for example, additive or multiplicative compared to stressors in isolation, or interactions may result in synergistic or antagonistic effects (Côté et al. 2016; Gunderson et al. 2016). As such, habitat-associated differences between individuals may lead to varied responses to noise disturbance.

In 2016, prolonged thermal stress due to high sea-surface temperatures along the Great Barrier Reef caused severe, widespread mass bleaching events and subsequent mortality of corals (Hughes et al. 2017). Many small-bodied coral reef fish are dependent on the three-dimensional structure provided by coral reefs for shelter when threatened, as nocturnal retreats and for food (Beukers et al. 1997; Froukh and Kochzius 2008; Pratchett et al. 2008; Graham et al. 2009). Degradation of these habitats can increase the susceptibility to predation and ultimately the risk of extinction for these dependent species (Pratchett et al. 2008; Coker et al. 2009; Boström-Einarsson et al. 2018). Despite declines in total fish abundances from such events (Wilson et al. 2006), fishes can persist on degraded habitats, albeit with potential sublethal consequences (Pratchett et al. 2004). Host-coral degradation has been shown to affect growth rates of damselfishes not reliant on coral as a food source, with increases in physiological stress as a possible mechanism (Feary et al. 2009). To our knowledge, no study has examined how such variation in habitat quality may influence the responses of coral-dwelling fishes to anthropogenic noise.

Here, using real motorboats for acoustic validity, we experimentally tested the influence of body condition and habitat quality on the responses of a common coral reef fish (the blue-green damselfish, *Chromis viridis*) to anthropogenic noise. First, we compared the behavioural and physiological responses of fish in relatively good and poor condition to motorboat noise and ambient sound (as a control). We predicted that poorer-condition fish would be more sensitive and

perform worse when exposed to motorboat noise than better-condition conspecifics. Second, in a multi-site experiment, we investigated the influence of habitat degradation on fish responses to motorboat noise using a physiological response measure. We predicted that fish living on degraded habitats would be more sensitive and, thus, respond more strongly to short-term motorboat noise than fish inhabiting healthy host corals, potentially due to habitat-associated stress affecting responses to additional challenges.

3.3 METHODS

3.3.1 Ethics statement

This research was performed in accordance with the Association for the Study of Animal Behaviour 'Guidelines for the treatment of animals in behavioural research and teaching', with permission and approval from: the Animal Welfare and Ethical Review Body (University of Bristol: UIN/13/036; University of Exeter: 2013/247); Lizard Island Research Station, Great Barrier Reef Marine Park Authority, James Cook University (A2081).

3.3.2 Experimental overview

This study was conducted during November–December 2016 at Lizard Island Research Station (LIRS) (14° 40' S, 145° 28' E), Great Barrier Reef, Australia. The study had two main parts. First, we assessed the responses to motorboat disturbance of fish categorised as being in 'good' or 'poor' body condition. Wild-caught fish were collected from inside the lagoon surrounding Lizard Island, transported back to LIRS, measured (length and mass) and kept overnight in holding tanks. The following day, fish were tested *in situ* in the lagoon using a physiological (opercular beat rate) and a behavioural (anti-predator startle) metric to investigate responses to either real-motorboat noise or ambient conditions. Second, we tested the response to real-motorboat noise of fish from host-corals classified as either healthy or degraded to determine whether previous environmental disturbance can influence the impact of noise. Fish were collected from multiple sites from the lagoon, brought back to LIRS for measurement, and kept overnight in holding tanks. They were tested the following day *in situ* in the lagoon, examining a physiological (opercular beat rate) metric in response to either real-motorboat noise or ambient sound conditions. All experiments were conducted on the blue-green damselfish (*Chromis viridis*), a damselfish that often lives in large shoals (>100 individuals) and that is commonly associated with

live corals (Feary et al. 2007; Froukh and Kochzius 2008). *C. viridis* communicate using a series of clicks between 500 and 1000 Hz during both agonistic and courtship interactions (Amorim 1996), with their hearing range likely covering the same frequency bandwidth. In addition, small motorised vessels produce significant noise below 1 kHz (Erbe 2013; Brooker and Humphrey 2016), overlapping with the vocal range of *C. viridis*.

3.3.3 Acoustic stimuli

In all experiments, fish were either exposed to real motorboats driven continuously 10–200 m from the experimental setup with various steering patterns, or they experienced ambient conditions (as a control). Five motorboats (each with 5 m long aluminium hulls and 30 hp Suzuki 2-stroke outboard motors) were used in each experiment to minimise pseudoreplication; motorboats were randomly allocated for use in individual trials. Representative recordings were taken of the ambient conditions, each motorboat used in the experimental trials, and the holding-tank conditions in the LIRS aquaria (where fish were held prior to trials), in both acoustic-pressure and monoaxial particle-motion domains.

Acoustic pressure was measured with a calibrated omnidirectional hydrophone (HiTech HTI-96-MIN with inbuilt preamplifier, manufacturer-calibrated sensitivity -164.3 dB re 1V/µPa; frequency range 0.002–30 kHz; calibrated by manufacturers; High Tech Inc., Gulfport MS) and monoaxial particle motion was measured using a calibrated triaxial accelerometer (M20-040; sensitivity 0–3 kHz; Geospectrum Technologies, Dartmouth, Canada) both connected to a digital 8-track field recorder (Zoom F8 field recorder, sampling rate 48 kHz, Zoom Corporation, Tokyo, Japan). All recording levels were calibrated using a 1 kHz pure sine wave signal of known voltage recorded in-line with an oscilloscope. For analysis, 20 s of motorboat passes from each boat (n = 5) used in the experiments were appended together into a single recording; 20 s from each of the eight different ambient recordings were similarly appended into a single recording; and a single 1 min holding-tank recording was used. All recordings were analysed with the PaPAM acoustics analysis package (Nedelec et al. 2016a) using MATLAB v2014a. The power spectral density of each recording was determined (Fig. 3.1); power spectral densities are presented over a frequency range of 0–3000 Hz as that is most appropriate for the likely hearing range of this species (see above).

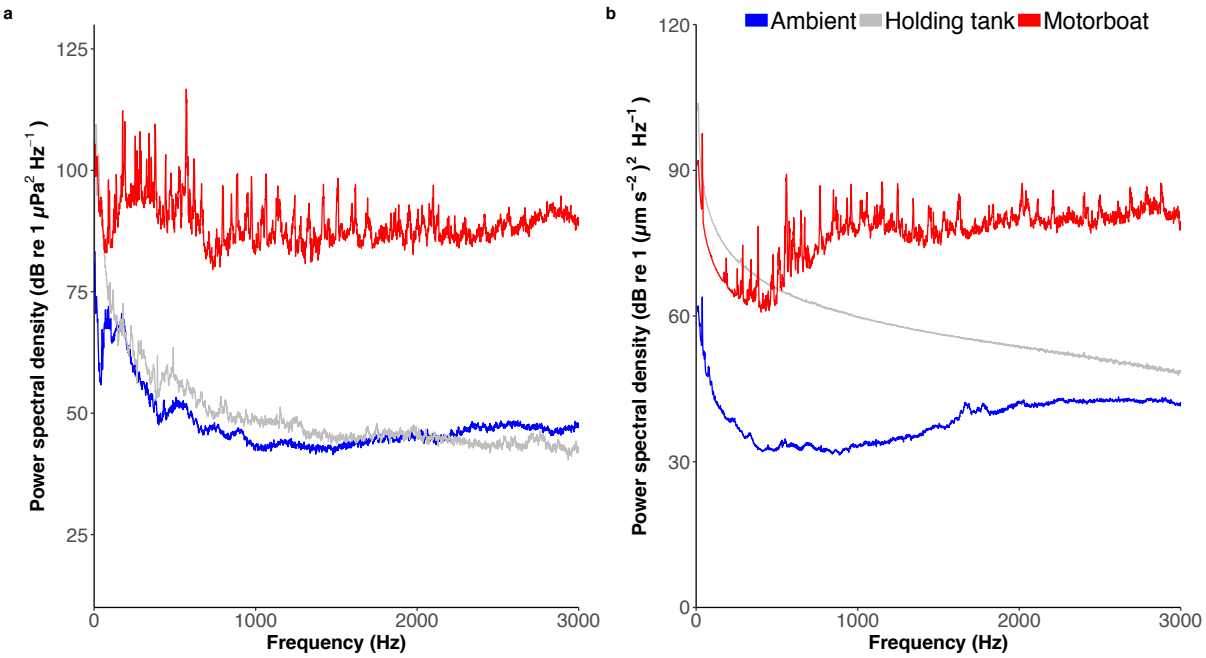

Figure 3.1. Analysis of acoustic conditions. Spectral content of ambient and motorboat field recordings, as well as the holding-tank conditions, measured in both (a) acoustic pressure and (b) particle motion. Mean power spectral density of all conditions are shown. Sounds analysed with the *PaPAM* acoustics analysis package (Nedelec et al. 2016a) using MATLAB v2014a; fft length = sampling frequency (48 kHz for ambient/motorboat noise, 44.1 kHz for holding-tank conditions), Hamming evaluation window, 50% window overlap.

3.3.4 Variation in response to motorboat noise due to body condition

Chromis viridis were collected by divers using a barrier net each day from a single site and transported to holding facilities at LIRS. The fish were measured (mass, to the nearest 0.01 g; standard length, to the nearest 0.1 cm) and overall body condition assessed using residual regression analysis (Koops et al. 2004). In this analysis, the position of each individual residual from the mass~length relationship is used as an indicator of relative body condition. The subsets of fish with the highest (33%) and lowest (33%) residuals ('good' and 'poor' condition, respectively) were placed into separate aquaria.

The day after capture, fish were transported in temporary holding containers to the testing sites approximately 0.4–1 km away. Transport was either on foot (approx. 5 min) or by kayak (approx. 20 min), to avoid unwanted exposure to vehicle or motorboat noise prior to testing. At the testing site, fish were held in 250 L temporary aquaria under shade cloth on the beach; the water

was refreshed regularly to maintain temperature and oxygen levels. Fish categorised as either 'good' or 'poor' condition were exposed to either ambient conditions or motorboat disturbance in an independent-measures design. Trials were conducted in a counterbalanced block design to avoid any treatment bias in the time of day or duration that fish were held in the temporary aquaria.

As a physiological indicator of susceptibility to motorboat noise, we used opercular beat rate (OBR), a secondary measure for stress (Barton 2002; Simpson et al. 2015; Purser et al. 2016). For each trial, two fish from each body-condition category were randomly selected and placed into separate 200 ml transparent chambers and positioned in water of 1–2 m depth. Fish were given a 10-min acclimation period, followed by another 5 min of ambient conditions (as a pre-testing period) and then a 5-min exposure period consisting of either further ambient conditions or motorboat disturbance. This enabled a pre-testing OBR from each individual to be established in ambient conditions and allowed for a change-based analysis for consideration of the effect of motorboat disturbance, thus controlling for differences in baseline OBR (as in: Purser et al. 2016; Radford et al. 2016b). Following a trial, fish were removed from their chambers and transferred into a post-experiment holding tank. At the end of the day, all fish were released back onto the reefs from which they had been collected.

All trials were recorded using video cameras (GoPro Hero 3 or 4) and videos were archived for later data analysis. The videos were cut into two 5-min segments (pre-testing or exposure period) per trial and analysed in random order without sound, with the observer (H.R.H.) blind to the acoustic treatment and to the trial period. Opercular beats per min of each fish was determined for the duration of each 5-min trial period, and fish activity level characterised using a 3-point ordinal scale—1 (no swimming), 2 (occasional swimming; periods of rest interspersed with bouts of swimming), 3 (constant swimming in the tube)—as per Purser *et al.* (2016). A 5-min mean pre-testing OBR was calculated for each fish, and then subtracted from the OBR of each minute in the exposure period to determine the change in OBR over the trial for each fish.

As a behavioural measure and assessment of likely fitness consequences of motorboat disturbance, the anti-predator response to a looming stimulus was assessed (Simpson et al. 2015, 2016a). For each trial, an individual fish was transferred into a 500 ml transparent pot that was held in place with a bungee cord on a concrete block positioned in water of 1–2 m depth. The stimulus consisted of a 73 cm PVC pipe with black end cap, which was fired towards the fish using a spear-gun rubber; the use of a rubber cushion at the end of the PVC pipe prevented the stimulus from hitting

the chamber (see Fig. 3.2 for a photo of the experimental setup). Fish were given a 5-min acclimation period to recover from the disturbance of transfer. Following this, there was a 2-min exposure period of either ambient conditions or motorboat disturbance. At the end of the exposure period, the looming stimulus was released and the behaviour recorded with a video camera (GoPro Hero 3 or 4). All videos were analysed without sound, rendering the experimenter (H.R.H.) blind to the acoustic treatment. The presence or absence of a startle response (rapid shift in body position or directional change in swimming trajectory between consecutive frames) was scored (Simpson et al. 2015, 2016a). In those trials where there was a startle response, the distance from the looming stimulus to the fish at the time of startle was calculated.

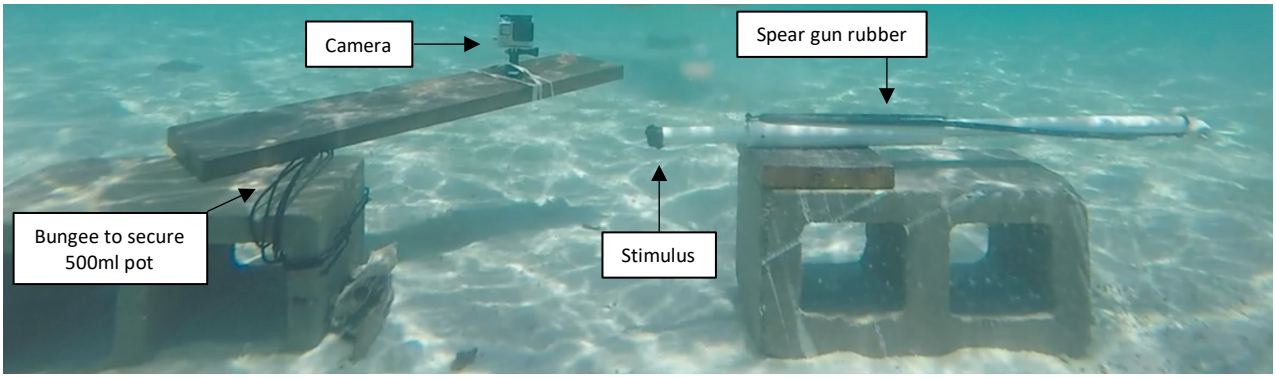

Figure 3.2. Image showing the setup for the looming-stimulus experiment. The 500 ml pot (absent from this photo) was held in place by the black bungee cord seen on the left-hand side of the image. The stimulus consisted of a 73 cm PVC pipe with black end cap (seen here, partly protruding from the larger PVC it was normally housed within). The GoPro camera in the background was used to record each trial. All equipment was weighted down by attachment to the two breeze blocks.

3.3.5 Multi-site comparison of fish associated with healthy or degraded host corals

To assess the influence of habitat quality on susceptibility to motorboat disturbance, *C. viridis* were collected from 10 coral reef sites inside the Lizard Island lagoon. Sites were classified as either 'healthy' or 'degraded' habitats depending on the percentage of live coral cover: healthy sites contained over 90% live coral cover, while degraded sites had less than 50% live coral cover (Fig. 3.3).

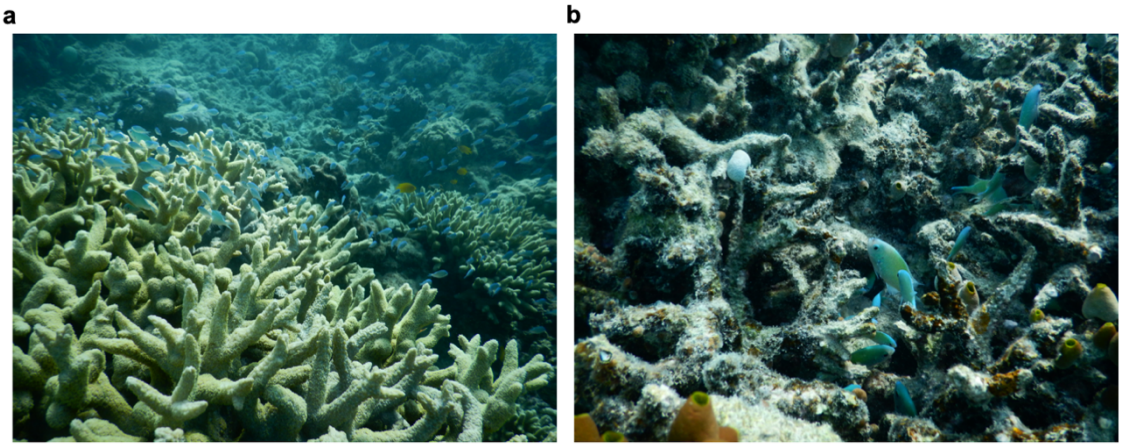

Figure 3.3. Representative examples of the two classified habitat types at Lizard Island (Great Barrier Reef, Australia). (a) Healthy coral with *Chromis viridis* swimming above and (b) degraded coral with sheltering *C. viridis*.

Fish were collected from each site by divers using a barrier net, and transported back to LIRS for measurement (mass, standard length; as above) and subsequently used in experimental trials the day after capture. The relative body condition of fish from the two habitat classifications was determined using residual regression analysis (Koops et al. 2004) as per the methodology in the body-condition experiment (see above). OBR was chosen for use as that method facilitated collection of a large sample size, and the habitat-quality experiment took place before analysis of the data from the body-condition experiment (so knowledge of those findings was not yet available). The experimental protocol followed that described above for the body-condition experiment. OBR (beats per minute) was determined from the first 2 min of each trial video (except in six cases). Pre-testing OBR was assessed for both habitat classifications, which was subsequently used as an indicator of underlying chronic stress levels. For each individual fish, pre-testing OBR was subtracted from that in the exposure period to allow a change-based analysis when considering the impact of motorboat noise.

3.3.6 Statistical analysis

Statistical analysis was carried out in R (Version 3.2.2). OBR data from the body-condition experiment were analysed with linear mixed models (LMMs), following visual assessment of the normality of residuals and assessment of homogeneity of variance. OBR data from the body-condition experiment were analysed with sound treatment (motorboat disturbance vs ambient

conditions), body condition (good vs poor) and time of exposure period (1–5 min), as well as the interaction between body condition and sound treatment, as fixed factors. Trial ID (corresponding to each individual fish, to account for the repeated-measures element with time in the trial) and fish activity level in the exposure period (1–3) were included as random terms. For the looming-stimulus experiment, the likelihood of fish exhibiting a startle response and the distance from stimulus at the point of startle were analysed with generalised linear models (GLM) with a binomial and gamma error distribution, respectively. Both models included sound treatment (motorboat disturbance vs ambient conditions), body condition (good vs poor) and their interaction as factors. Residual diagnostic plots were checked using the DHARMa package in R (Hartig 2017). OBR data from the habitat-quality experiment were analysed using a LMM with sound treatment (motorboat disturbance vs ambient conditions) and habitat designation (healthy vs degraded) and their interaction as fixed factors; site (1–10) and fish activity level in the exposure period (1–3) were included as random terms. In all analyses, results were determined by comparisons excluding the term of interest; all post-hoc tests were performed using the emmeans package in R (Lenth 2018).

3.4 RESULTS

3.4.1 Variation in response to motorboat disturbance due to body condition

In the pre-testing period, fish in poor body condition had a significantly higher opercular beat rate (OBR) than fish in good condition (Welch two-sample t-test: $n_{poor} = 59$, $n_{good} = 52$, $t = -2.07$, $df = 108.84$, $p = 0.04$; Fig.3.4).

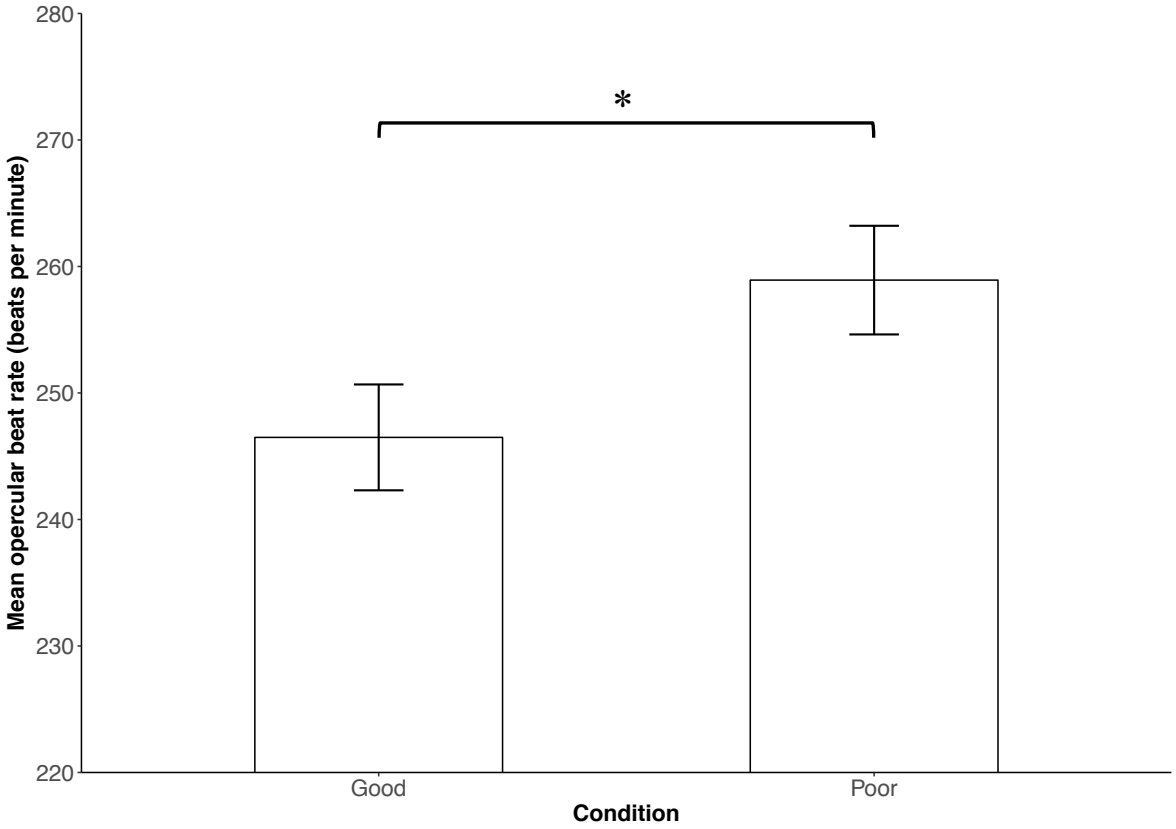

Figure 3.4. Pre-testing opercular beat rate of fish in different body conditions. Mean ± SE raw pre-testing opercular beat rate from fish classified in good and poor condition (n_{good} = 52, n_{poor} = 59). * = $p < 0.05$.

The change in OBR from pre-testing to exposure period was not significantly affected by sound treatment (LMM: $\chi^2 = 0.0002$, df = 1, p = 0.99), body condition ($\chi^2 = 0.03$, df = 1, p = 0.86) or their interaction ($\chi^2 = 0.08$, df = 1, p = 0.78); there was also no difference in the change in OBR over the 5-min exposure period ($\chi^2 = 1.99$, df = 4, p = 0.74; Fig. 3.5; see Appendix B, Table B.1 for the full model output).

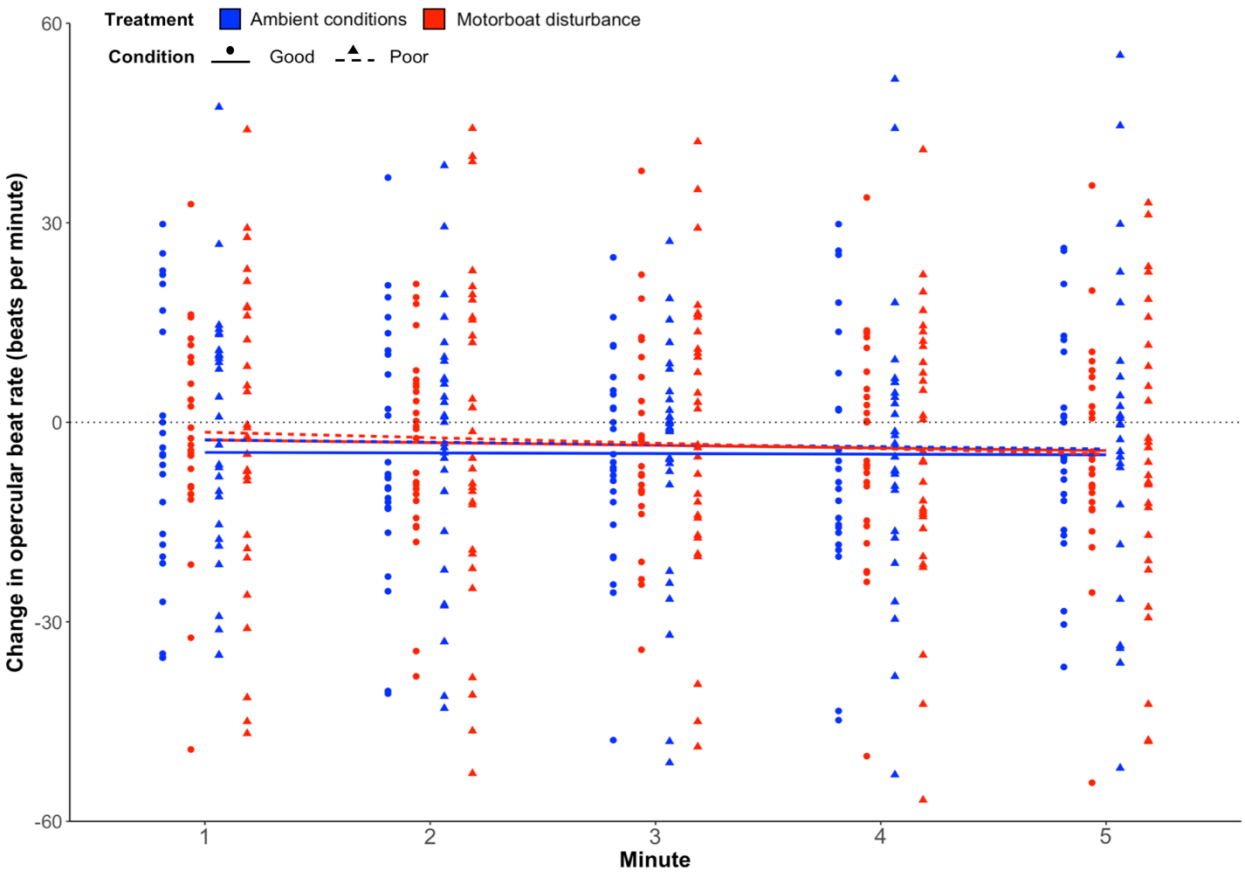

Figure 3.5. Physiological response of fish in different body condition to motorboat disturbance and ambient conditions. Change in opercular beat rate (from pre-testing to exposure period) for each minute in the 5-min exposure period, separated by sound treatment and body condition ($n_{ambient\text{-}good}$ = 25, $n_{motorboat\text{-}good}$ = 27, $n_{ambient\text{-}poor}$ = 29, $n_{motorboat\text{-}poor}$ = 30). The interaction between sound treatment and body condition was not significant (χ^2 = 0.08, df = 1, p = 0.78); nor was there a difference in the change in opercular beat rate over the 5-min exposure period (χ^2 = 1.99, df = 4, p = 0.74).

The likelihood that fish startled to the looming stimulus was not significantly affected by sound treatment (GLM: χ^2 = 1.33, df = 1, p = 0.25), body condition (χ^2 = 0.70, df = 1, p = 0.40) or their interaction (χ^2 = 1.39, df = 1, p = 0.24; Fig. 3.6a; Appendix B, Table B.2). However, for fish that did startle, the distance from the looming stimulus at the onset of the startle response was significantly affected by the interaction between sound treatment and body condition (χ^2 = 1.08, df = 1, p = 0.04; Fig. 3.6b; Appendix B, Table B.3). Fish in good and poor condition startled at similar distances to the looming stimulus in ambient conditions (Tukey's pairwise comparison: p = 1.00) but, during motorboat disturbance, fish in poor body condition only startled when the looming stimulus was significantly closer than was the case for fish in good condition (p = 0.03).

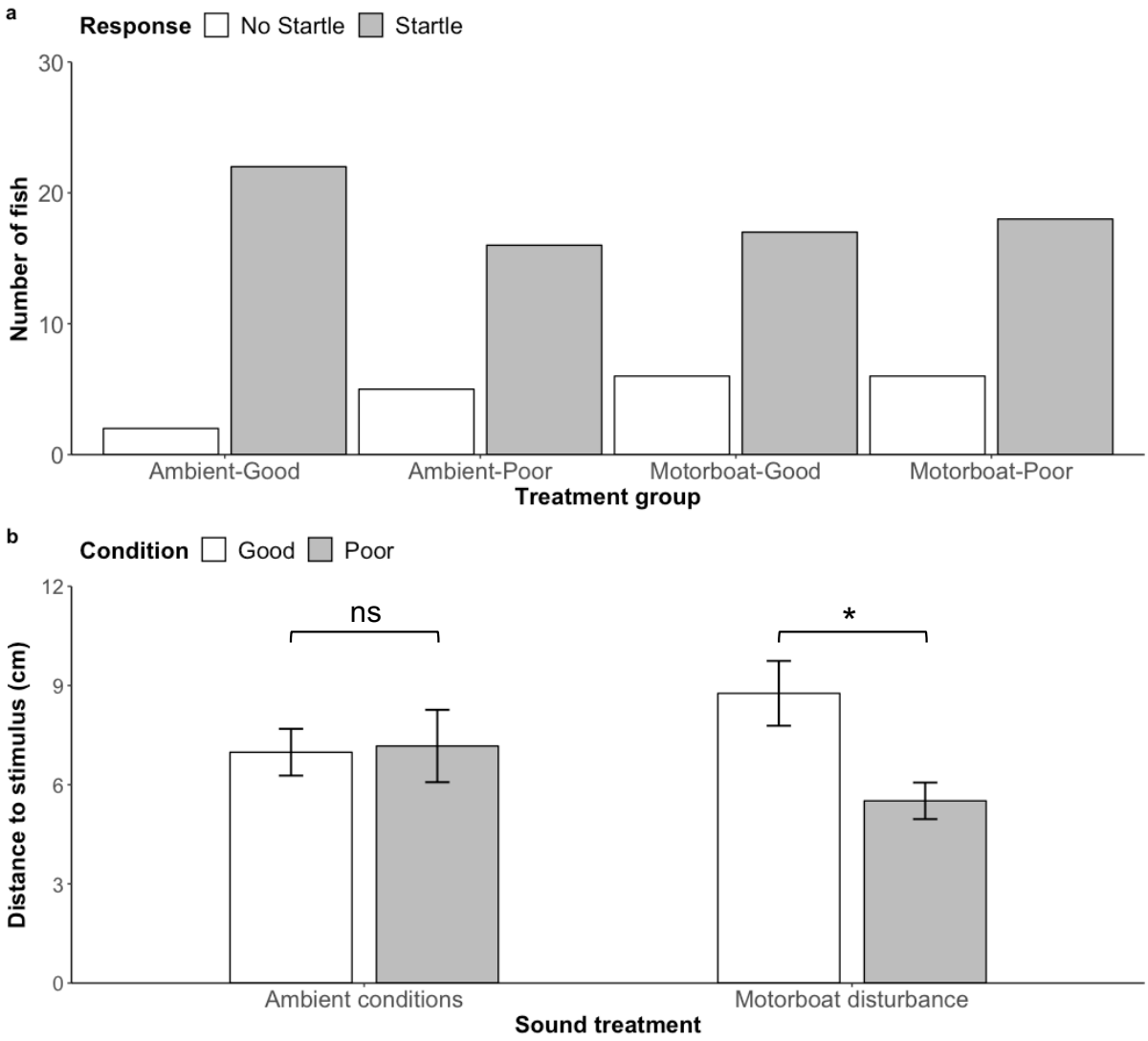

Figure 3.6. Behavioural responses of fish in different body condition to motorboat disturbance and ambient conditions. (a) Number of *C. viridis* which displayed a startle response or did not startle to the looming stimulus ($n_{Ambient–Good}$ = 24; $n_{Motorboat–Good}$ = 23; $n_{Ambient–Poor}$ = 21; $n_{Motorboat–Poor}$ = 24). The likelihood that fish startled to the looming stimulus was not significantly affected by sound treatment (GLM: χ^2 = 1.33, df = 1, p = 0.25), body condition (χ^2 = 0.70, df = 1, p = 0.40) or their interaction (χ^2 = 1.39, df = 1, p = 0.24). (b) Mean ± SE distance to the stimulus when the startle occurred ($n_{Ambient–Good}$ = 22; $n_{Motorboat–Good}$ = 17; $n_{Ambient–Poor}$ = 16; $n_{Motorboat–Poor}$ = 18). ns = no significance; * = $p < 0.05$.

3.4.2 Multi-site comparison of fish associated with degraded or healthy host corals

To assess whether body condition may be a potential mechanism for any differences in responses observed between fish from the two habitat classifications, relative body condition was determined

from the total sampled population using the mass~standard length relationship (Fig. 3.7). Fish with negative and positive residuals were classified as being in poor and good condition, respectively. Fish body condition, pooled from the total sample collected across all sites, did not differ significantly between healthy and degraded habitats (Welch two-sample t-test: t = 0.20, $n_{healthy}$ = 221, $n_{degraded}$ = 220, df = 381.73, p = 0.84; Fig. 3.7).

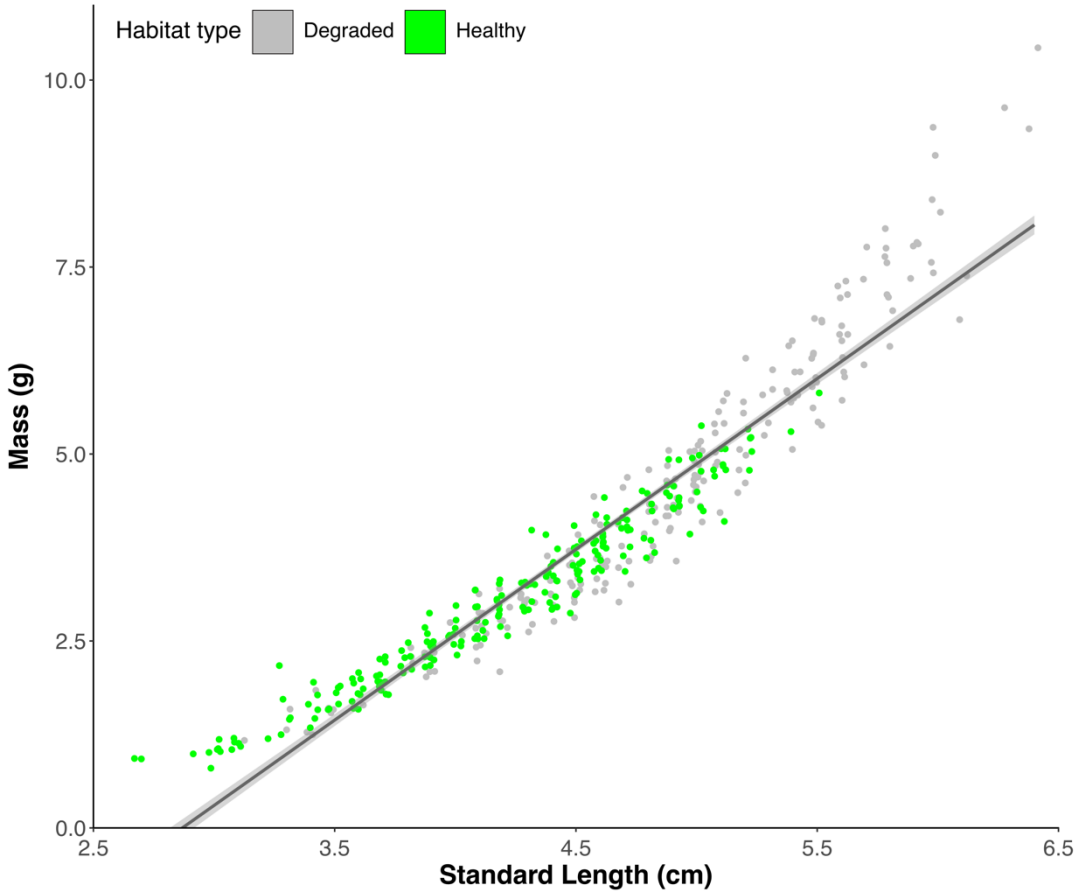

Figure 3.7. The relationship between mass and standard length for fish from healthy and degraded host corals. The residuals from the linear regression line were used to determine relative body condition, as per the methodology of Koops et al. 2004. For fish from degraded sites, 56.4% and 43.6% were categorised into negative and positive residuals respectively; from sites classified as healthy, 55.7% had negative residuals and 44.3% had positive residuals.

In the pre-testing measurement period, OBR was significantly higher in fish from healthy habitats than degraded sites (Welch two-sample t-test: t = -2.70, $n_{degraded}$ = 127, $n_{healthy}$ = 122, df = 232.51, p = 0.01; Fig. 3.8).

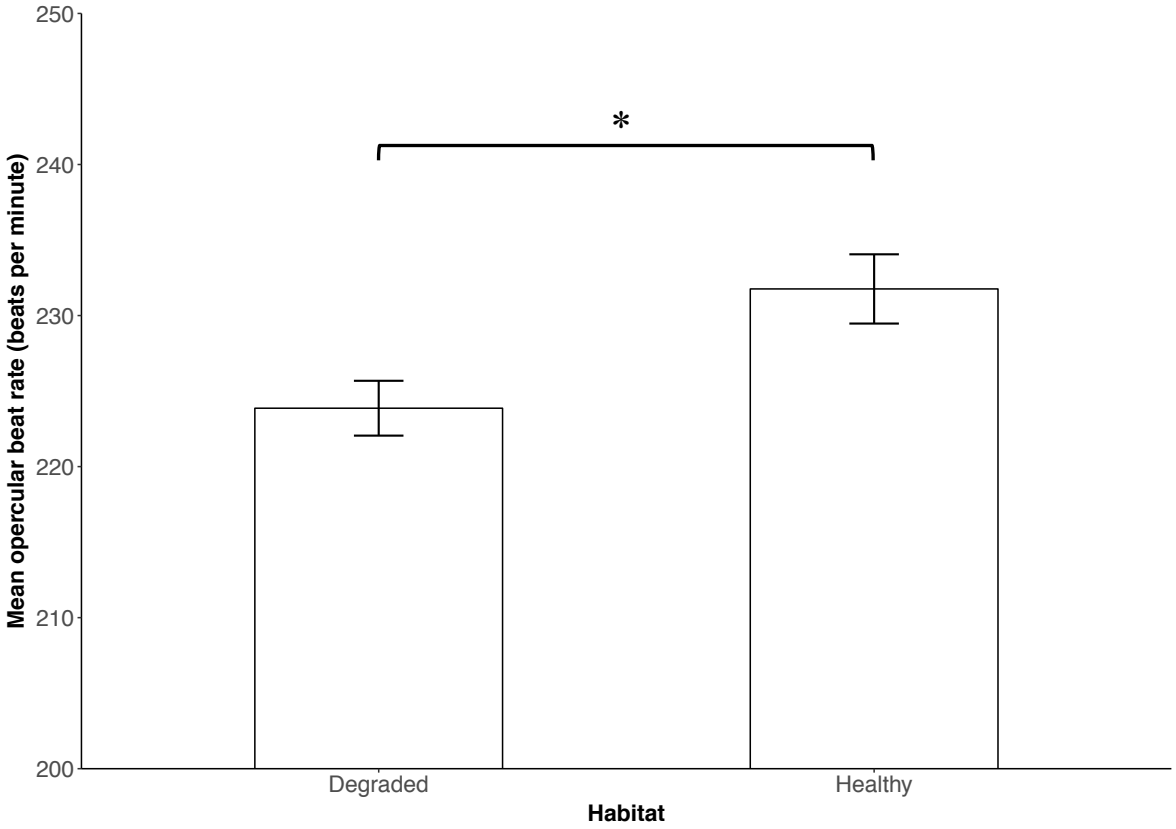

Figure 3.8. Mean ± SE pre-testing opercular beat rate of fish from degraded and healthy habitats ($n_{degraded}$ = 127, $n_{healthy}$ = 122). * = $p < 0.05$.

The change in OBR (from pre-testing to exposure period) was significantly affected by the interaction between sound treatment and habitat designation (LMM: $\chi^2 = 4.56$, df = 1, p = 0.03; Fig. 3.9; Appendix B, Table B.4). However, *a priori* contrasts revealed no significant differences between ambient conditions and motorboat disturbance for fish from degraded habitats (Post-hoc contrast: t-ratio = 1.65, df = 243, p = 0.10), as well as no significant difference between the two sound treatments from fish from healthy habitats (t-ratio = - 1.38, df = 243, p = 0.17).

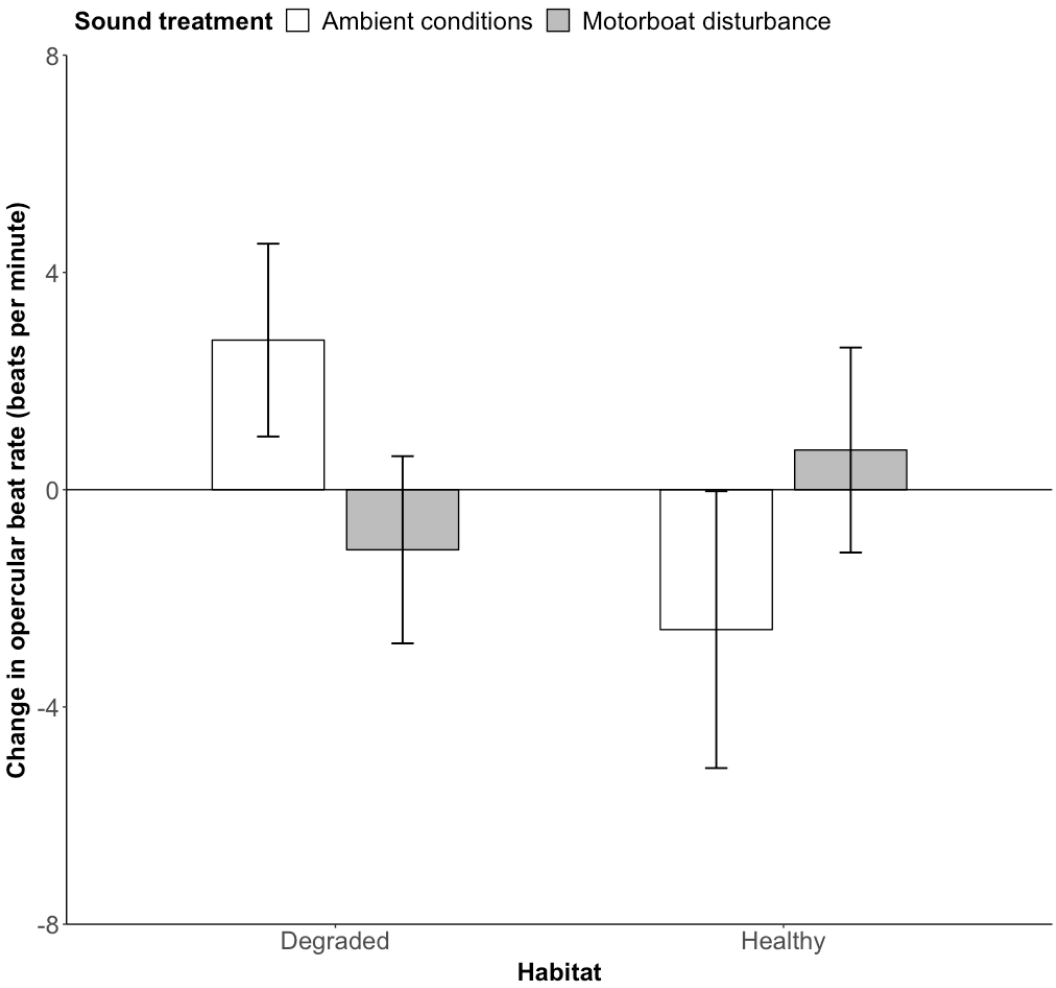

Figure 3.9. Change in opercular beat rate (OBR) of fish from different habitat classifications, exposed to either ambient conditions or motorboat disturbance. Mean ± SE change from pre-testing to exposure period ($n_{degraded\text{-}ambient}$ = 61, $n_{degraded\text{-}motorboat}$ = 66, $n_{healthy\text{-}ambient}$ = 59, $n_{healthy\text{-}motorboat}$ = 63). Change in OBR was significantly affected by the interaction between sound treatment and habitat designation (LMM: χ^2 = 4.56, df = 1, p = 0.03).

3.5 DISCUSSION

This study presents the first *in situ* experiments testing the influences of fish body condition and habitat quality on responses to motorboat noise. We found that, when exposed to motorboat noise, *C. viridis* in poorer body condition only startled to a looming stimulus when it was closer to them compared to individuals in better condition; there was no body-condition difference in the distance to the stimulus when the startle occurred in ambient conditions. This finding corroborates the results of a previous laboratory-based playback study on European eels (Purser et al. 2016), but uses real motorboats in open-water conditions for acoustic validity. We found no significant effects of

motorboat noise or its interaction with body condition on a physiological metric, opercular beat rate (OBR). We also found no significant difference in OBR of fish from degraded vs healthy reef sites when exposed to motorboat noise. Although, we found a significant overall interaction between sound treatment and habitat type, post-hoc comparisons did not reveal any significant effects. Such experiments are important to expand our understanding beyond just documenting that noise has an effect to considering intraspecific variation (Radford et al. 2016a; Harding et al. 2019; Chapter 1).

We found pre-testing OBR to be significantly higher in fish in poorer body condition than those in better condition, which may underpin the documented difference in behavioural response to noise. First, the elevated pre-testing OBR seen here may be indicative of underlying chronic stress in the poorer-condition fish. Elevated stress can affect cognitive functioning (e.g. causes lapses in attention; Mendl 1999), which may explain the reduced anti-predator performance in poorer-condition fish under stressful conditions. Heightened physiological stress can also reduce the available energy to respond appropriately, if at all, to additional challenges (Schreck and Tort 2016). Second, individuals are only able to process a finite amount of information (Washburn and Taglialatela 2012), which may be further reduced in poorer-condition fish, and the distracted-prey hypothesis suggests that noise can occupy some of the available processing ability and thus negatively affect predator-avoidance behaviour (Chan et al. 2010). Third, a wide range of fishes have shown state-dependent behaviours, where those with reduced energy reserves are more likely to take risks in order to continue provisioning (Näslund and Johnsson 2016). When in a low energetic state (e.g. starved), an individual might display risk-prone behaviours when confronted by an additional stressor in order to continue foraging despite the increased risk of predation (Biro and Booth 2009; Sih and Del Giudice 2012).

The lack of a significant effect of motorboat noise on fish responses from either habitat type may be due to underlying fish body condition. We predicted that underlying fish condition, as a result of stress-associated habitat disturbance, may represent a potential mechanism for any differences observed, with condition previously shown to affect sensitivity to noise (Purser et al. 2016; this chapter). However, we found no underlying differences in fish body condition between the two habitat types. Similarly, two other species of Pomacentrids (*Chrysiptera parasema* and *Dascyllus melanurus*) monitored over 29 days on degraded and healthy habitats showed no differences in condition (Feary et al. 2009). We only used a physiological metric to test for habitat-associated variation in responses to motorboat noise. If we had conducted the analysis of the body-condition experiment before this one, we would have used the behavioural metric as well; however,

these data were collected in a single season with no time for analysis whilst in the field. Other studies have found effects of coral-reef habitat degradation on behaviour (McCormick 2012; Boström-Einarsson et al. 2018). Future experiments, therefore, could test the behavioural responses of free-swimming coral-dwelling fishes to motorboat disturbance at their host coral colony.

Assessment of pre-testing OBR revealed higher rates in fish from healthy habitats compared to degraded coral reefs, which is contrary to our expectations. Recent mass-bleaching events and cyclones experienced by the Northern Great Barrier Reef have resulted in significant habitat degradation (Hughes et al. 2017; Gordon et al. 2018). Live coral habitats are, therefore, becoming increasingly sparse. Given that many juvenile coral reef fish recruit to live coral, and adults relocate to live coral heads if given the opportunity (Coker et al. 2012a,b), healthy habitats could become increasingly crowded. Such crowding could lead to increased intraspecific competition for space and possible concomitant increases in stress levels (Coker et al. 2012b). This may explain the higher pre-testing OBR in fish from healthy habitats, although we were unable to get density estimates of fish from each habitat type.

The increase in OBR in poor-condition fish seen in Purser *et al.* (2016) was in contrast to our findings, where we found no such changes in *C. viridis* exposed to real motorboats in either the body-condition or habitat-quality experiments. OBR in fish has been shown to be affected by anthropogenic noise in a number of other species (Simpson et al. 2015; Bruintjes et al. 2016b; Nedelec et al. 2016b). As such, we would have predicted similar increases in OBR for *C. viridis* given the behavioural response, and the likely hearing ability of *C. viridis*; this species produces a series of clicks between 500 and 1000 Hz (Amorim 1996), with hearing likely to cover the same frequency bandwidth. It is possible that the fish in our study had high transport stress and thus were already at a maximum OBR, so when presented with the additional stress of passing motorboats were unable to respond with further increases in OBR.

These experiments add to the small, but increasing body of work investigating intraspecific variation in responses to anthropogenic noise (see Fig. 1.2; Chapter 1). Considering intraspecific variation in noise responses is vital in order to develop a full understanding across all levels of biological organisation and prevent misinterpretations that may lead to over- or under-estimation of the impacts. Intraspecific variation influences population dynamics, community structure and ecosystem functioning (Post et al. 2008; Des Roches et al. 2017). Thus, failure to consider the variation that exists within a species greatly undermines our ability to determine the full impacts of

this pervasive pollutant. The body condition of individual fish within a population can vary spatially and temporally, with decreases likely in, for instance, spawning periods (Dutil and Lambert 2011; Brosset et al. 2015). Exposure to anthropogenic noise at these times would likely prove more detrimental to these individuals than at other times when they may be in better condition. As our understanding of the impacts of anthropogenic noise increases, mitigation strategies and management decisions should ensure all individuals of a population are protected.

Chapter 4 – Fish in habitats with higher motorboat disturbance show reduced sensitivity to motorboat noise

Contributions to the work

H.R.H., T.A.C.G. (Imperial College London Masters student; Masters project on anthropogenic noise with in-field supervision by H.R.H, and acted as field assistant) and supervisors A.N.R. and S.D.S. conceived the research and designed the experiments. H.R.H., T.A.C.G., R.E.H. (Unaffiliated colleague; field assistant) and A.C.E.M (University of Bristol Undergraduate; field assistant). conducted the fieldwork. H.R.H conducted the analyses and interpreted the results, aided by T.A.C.G. and advised by A.N.R and S.D.S. H.R.H. drafted the chapter, with comments provided by T.A.C.G., A.N.R. and S.D.S.

The physiological elements of this chapter have been published as:
Harding HR, Gordon TAC, Hsuan RE, Mackaness ACE, Radford AN, Simpson SD. 2018. Fish in habitats with higher motorboat disturbance show reduced sensitivity to motorboat noise. *Biol. Lett.*, 14, 20180441.

4.1 ABSTRACT

Anthropogenic noise can negatively impact many taxa worldwide. However, many studies have been conducted in captivity and/or with playback of sounds; research in natural conditions with real-world sound sources are important. Moreover, it is possible that the range and severity of impacts could diminish over time in noisy, high-disturbance environments, but the influence of previous disturbance remains untested in natural conditions. This study demonstrates effects of short-term motorboat noise on the behaviour and physiology of an endemic cichlid fish in Lake Malaŵi. Loudspeaker-playback of motorboat noise caused behavioural changes at a single lower-disturbance site, characterised by low historic and current motorboat activity; noise-exposed fish displayed an increased number of aggressive chases compared to control fish. Exposure to motorboats (driven 20–100 m from fish) and loudspeaker-playback of motorboat noise both elevated oxygen-consumption rate at the same site. Repeating this physiological assay at further lower-disturbance sites revealed a consistent effect of elevated oxygen consumption in response to motorboat disturbance. However, when similar trials were conducted at four higher-disturbance sites (with greater historic and current motorboat activity), no effect of short-term motorboat exposure was detected. These results demonstrate that disturbance history can affect local population responses to short-term noise exposure. Action regarding noise pollution should consider the past, as well as the present, when planning for the future.

4.2 INTRODUCTION

Anthropogenic noise is present in many biomes across the planet, elevating overall acoustic energy and creating noises that are characteristically different from naturally occurring sounds (Hildebrand 2009; Buxton et al. 2017). Recent work has demonstrated that noise pollution can have a wide range of physiological and behavioural effects on many taxa (for recent reviews, see: Shannon et al. 2015; Kunc et al. 2016). Consequently, anthropogenic noise is considered a global pollutant that appears in international legislation, including the European Commission Marine Strategy Framework Directive and the US National Environment Policy Act.

To date, most studies investigating the consequences of anthropogenic noise for animals have used response means to test for overall impacts on a cohort of individuals, whilst largely ignoring the variation around the mean which may be driven by intrinsic characteristics or extrinsic factors (Radford *et al.* 2016a; Chapter 1). However, individual responses within a generation can be

affected by prior experience; for example, organisms might exhibit altered tolerance through habituation, sensitisation or hearing threshold shifts, or emigrate because of past disturbances (see Scholik and Yan 2002; Bejder *et al.* 2009; Radford *et al.* 2016b). Furthermore, population responses may be altered over multiple generations through evolutionary adaptation (Luther and Derryberry 2012). Experimental manipulations on captive animals have shown that repeated exposure to anthropogenic noise can alter short-term responses in several species (Nedelec et al. 2016b; Radford et al. 2016b). However, studies are lacking that explore how natural variation in responses are related to the long-term disturbance history of wild populations (see Bejder et al. 2009; Lowry et al. 2011).

Motorboat noise is increasing globally (Whitfield and Becker 2014), and has a range of detrimental behavioural, physiological and fitness impacts on fishes (Kunc et al. 2016; Simpson et al. 2016b). However, non-uniform distributions of boat use across space and time mean that fishes are exposed to varying levels of motorboat activity (Hildebrand 2009). Here, we investigate how the impact of motorboat noise on a wild endemic cichlid in Lake Malaŵi is affected by variation in disturbance history. First, at a single site with low historic and current motorboat counts, we tested behavioural responses to *in situ* loudspeaker playback of motorboat noise and an ambient-sound playback control, and a physiological response (oxygen consumption) to *in situ* exposures of both real motorboats driven around the testing site (hereafter referred to as 'motorboat disturbance') and loudspeaker playback. The effect of motorboat disturbance on oxygen consumption was then tested at three further lower-disturbance sites. Finally, we used the same physiological assay to test the response of fish to motorboat disturbance at four higher-disturbance sites (where there was higher historic and current motorboat activity).

4.3 METHODS

4.3.1 Ethics and permits

This research was performed in accordance with the Association for the Study of Animal Behaviour 'Guidelines for the treatment of animals in behavioural research and teaching', Malaŵian legal requirements, and institutional approval by the Animal Welfare and Ethical Review Body (University of Bristol: UIN/13/036; University of Exeter: 2013/247), with official permission from the Department of National Parks and Wildlife, Malaŵi.

4.3.2 Study system and sites

Work was conducted during April–July 2016 at Thumbi West Island, Lake Malaŵi (14° 01' S, 34° 49' E). Motorboat activity on Lake Malaŵi shows considerable spatial variability, with total usage likely to increase in the near future as a result of both human population increase (Ministry of Development Planning and Cooperation 2010) and development of fishing and tourism industries (Allison and Mvula 2002; Makochekanwa 2013). Adult males of the endemic cichlid *Cynotilapia zebroides* (previously known as *C. afra* and *Microchromis zebroides*) were chosen as the study organism based on: its abundance at the study site; its distinctive colouration allowing accurate identification despite the high species diversity and multiple intra-population colour polymorphisms present in the Lake Malaŵi species complex (Turner et al. 2001; Streelman et al. 2004); and previous evidence that acoustic cues play important roles in the ecology of similar cichlid species (Bertucci et al. 2010; Bertucci et al. 2012). *C. zebroides* at Thumbi West Island represent a rapidly evolving population that are hybridising with the local *Metriaclima zebra* population (Streelman et al. 2004); this is the case for all studied fish at all experimental sites.

Sites were classified for disturbance levels based on analysis of both historic and current motorboat activity. Trip logs from both of the only two local dive operators (*Cape Maclear SCUBA* and *SCUBA Shack*), which represented all formally logged motorboat use in the area, were used to identify four lower-disturbance sites (no recorded visits in the last 3 years) and four higher-disturbance sites (visited in more than 8 of the last 12 months; mean ± SE, 51 ± 13 logged dives in 33 months) around the island. Combined visits to the four higher-disturbance sites comprised over 60% of total logged motorboating activity, and none of the four lower-disturbance sites ever appeared in either log. These logs provided a quantifiable and representative assessment of general patterns of local near-shore motorboat traffic by a much larger fleet of similar, unlogged vessels, since other motorboat users follow the leads of these dive operators when taking tourists to see fishes.

Counts of all vessel traffic confirmed that current motorboating activity matched historic patterns. Counts of current activity included all motorboat traffic in the area passing within 50 m of the selected sites (distance estimated visually; at least two counts per site with a total time of at least 4 h). Higher-disturbance sites (mean ± SE: 3.58 ± 0.82 boats/hr) experienced 10 times more passes per hour on average than lower-disturbance sites (0.37 ± 0.19 boats/hr), and the passes were over twice as close to shore (mean ± SE, higher-disturbance sites: 19 ± 1.4 m; lower-disturbance sites: 45 ± 1.6 m). Boat counts were made between 10:30 and 17:00, with no difference in times

between higher- and lower-disturbance sites. All selected sites were acoustically independent, shallow (2–5 m) coastal areas (6–13 m from shore) with a rocky benthic substrate, separated by a body of water with a minimum distance of 300 m and/or a prominent headland (see Fig. 4.1 for the locations of the eight sites around Thumbi Island West).

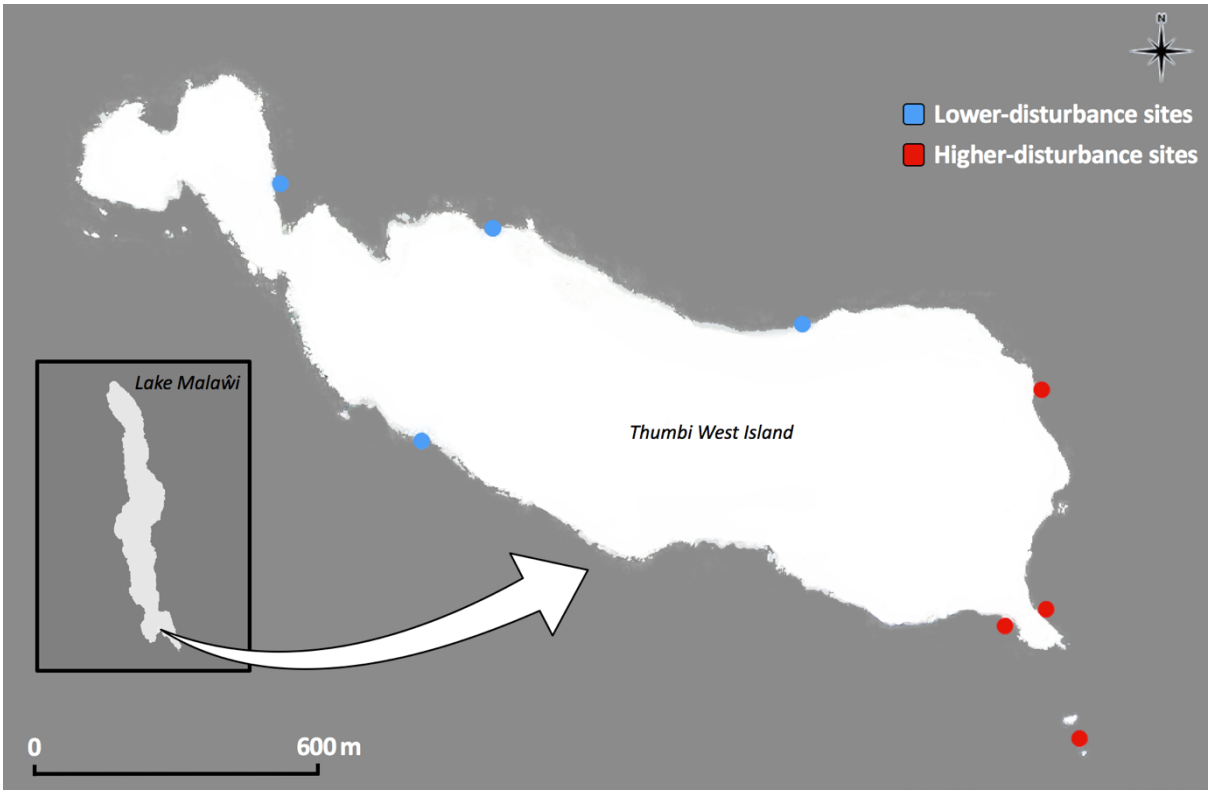

Figure 4.1. Image showing the location of the eight experimental sites around *Thumbi West Island* (14° 1’ 14” S, 34° 49’ 9” E). All sites were matched for benthic substrate (rocky bottom), depth (2–5 m), distance to shore (6–13 m) and mean water temperature (23.8–25.3°C).

4.3.3 Acoustic stimuli

For playbacks, 10 independent 5-min underwater recordings of daytime ambient conditions (five different times of day) and motorboat noise (five different boats) were taken at the initial lower-disturbance site. Recordings were taken in 2 m depth, 10 m from the shore, and ambient conditions had no boat traffic within 500 m. All tracks for playback experiments were created using Audacity 2.1.2 (www.audacityteam.org), and adjusted to ensure that the root-mean-squared average amplitude levels (used as a measure of track volume and analysed using SASLabPro v5.2.07; Avisoft Bioacoustics) received during trials were equivalent to those received in original recordings, as per methods in Simpson et al. (2015). The sound system for playbacks consisted of a loudspeaker (University Sound UW-30; maximal output 156 dB re 1 µPa at 1 m, frequency response 0.1–10 kHz;

Lubell Labs, Columbus, OH) positioned 3 m from the trial, an amplifier (M033N, 18 W, frequency response 0.04–20 kHz; Kemo Electronic GmbH, Germany), an MP3 player (BUSH, New Mexico, USA), and a battery (12v 12Ah sealed lead-acid; CSB Co. Ltd, Vietnam). Representative recordings of ambient sounds and motorboats were made at all sites for the multi-site comparison in 1.5–3 m depth, 6–13 m from shore, at the location of the fish during the trials. For the actual motorboat disturbance exposures, eight different boats were used across the eight sites (1–5 boats per site). Motorboats were all of the type most commonly used in the area (7-m-long wooden hulls with 8–15 horsepower rear-mounted outboard engines), driven at various speeds 20–100 m from the recording equipment, as per Simpson et al. (2016a).

Acoustic pressure was measured with a calibrated omnidirectional hydrophone (HiTech HTI-96-MIN with inbuilt preamplifier, manufacturer-calibrated sensitivity -164.3 dB re 1V/µPa; frequency range 0.002–30 kHz; calibrated by manufacturers; High Tech Inc., Gulfport MS) connected to a digital recorder (PCM-M10, 48 kHz sampling rate, Sony Corporation, Tokyo, Japan). Particle motion was measured with a calibrated accelerometer (M20L; sensitivity following a curve over the frequency range 0–2 kHz; calibrated by manufacturers; Geospectrum Technologies, Dartmouth, Canada) connected to a digital 4-track recorder (Boss BR-800, 44.1 kHz sampling rate, Roland Corporation, Los Angeles, CA) and expressed as acceleration. Recording levels were calibrated using 1 kHz pure sine wave signals from a function generator with a measured voltage recorded in-line on an oscilloscope. Sound-recording equipment was positioned 1 m above the lake bed attached to an inflatable raft or a submerged stand to avoid unwanted noise from waves on the hull of a rigid boat. The accelerometer was suspended by rope to reduce mechanical noise affecting the particle-motion recordings.

All recordings were analysed in both sound-pressure and particle-motion domains using MATLAB 2013a (MathWorks Inc., *PAMGuide* & *paPAM* analysis packages (Merchant et al. 2015; Nedelec et al. 2016a). Power spectral densities were determined across the likely hearing range of cichlids; this was estimated as being the frequency range 0–2 kHz, based on previous studies involving electrophysiological measurements of hearing ability (Kenyon et al. 1998; Maruska et al. 2012; Ladich and Schulz-mirbach 2013) and analysis of the frequency of courtship-associated cichlid vocalisations (Amorim et al. 2004, 2008; Maruska et al. 2012). Playback using loudspeakers alters the characteristics of the original recordings, but analyses of spectral content and sound levels showed that many of the characteristics of the original recordings were retained in playback, and that these characteristics differed between playbacks of ambient sound and motorboat noise (Fig. 4.2).

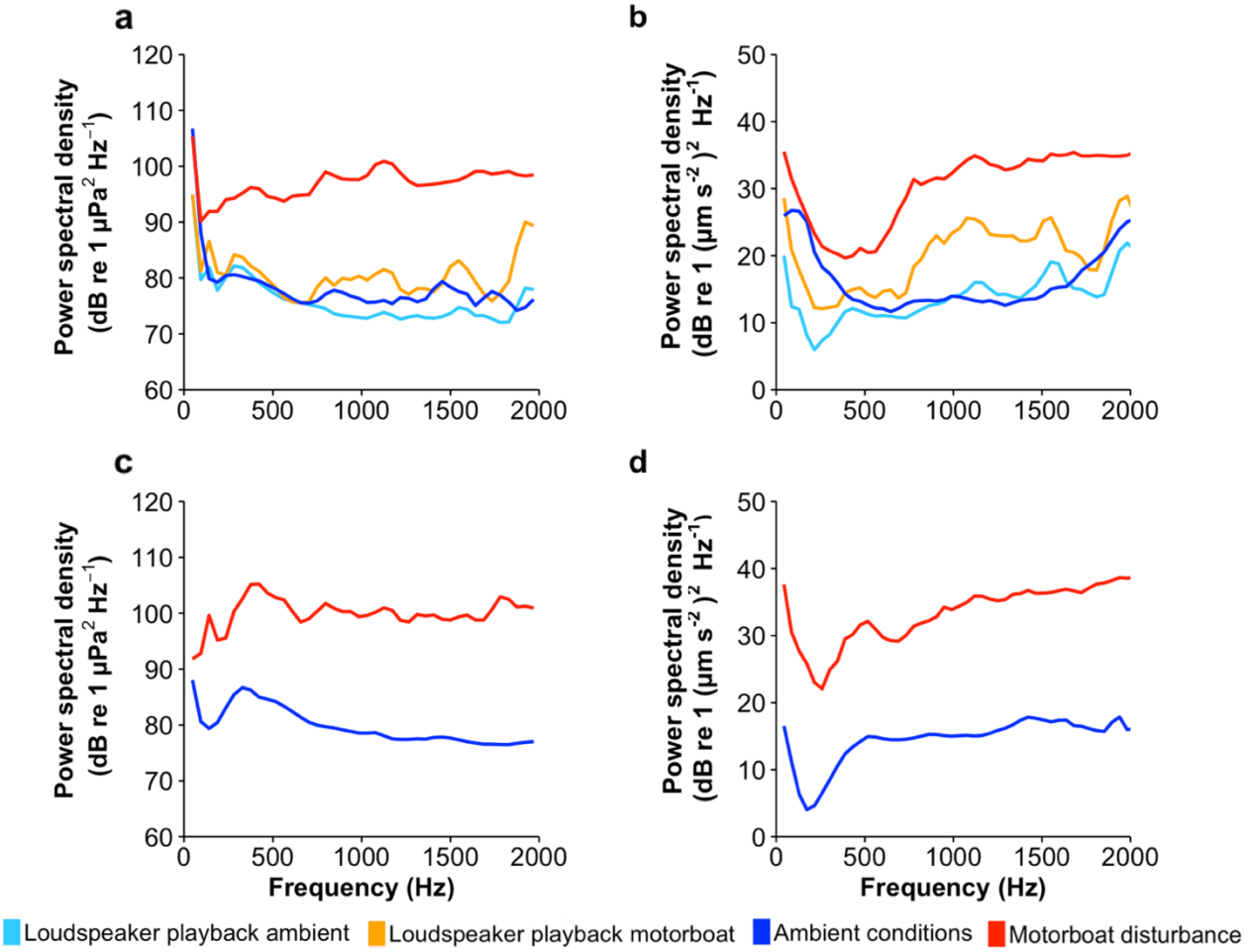

Figure 4.2. Acoustic analyses of experimental conditions. (a & b) Mean spectral content of combined illustrative recordings of original ambient conditions and motorboat noise, and playback of those recordings in both (a) pressure and (b) particle-motion domains, recorded at the initial lower-disturbance experimental site. Root-mean-squared sound-pressure levels (0–2 kHz) and range (given in dB re 1 µPa) are: 115.1, 113.3–116.4 (Ambient playback); 117.9, 112.4–121.2 (Motorboat playback); 125.1, 115.4–128.0 (Ambient); and 132.2, 127.5–134.9 (Motorboat disturbance). Particle-motion levels (0–2 kHz) and range (given in dB re (1 µm/s^2)) are 48.7, 44.6–52.9 (Ambient playback); 55.9, 43.8–62.4 (Motorboat playback); 53.6, 46.7–55.6 (Ambient); and 67.1, 64.1–69.0 (Motorboat disturbance). (c & d) Mean spectral content of combined illustrative recordings of original ambient conditions and motorboat noise taken at each subsequent site, in both (c) pressure and (d) particle-motion domains. Fast Fourier Transform (FFT) analysis of 0–2 kHz, spectrum level units averaged from 30 s recordings, Hamming evaluation window, FFT size = 1024. Root-mean-squared sound-pressure levels (0–2 kHz) and range (given in dB re 1 µPa) are: 115.5, 109.8–120.6 (Ambient); and 135.0, 120.8–142.4 (Motorboat disturbance). Particle-motion levels (0–2 kHz) and range (given in dB re (1 µm/s^2)) are: 49.1, 46.6–52.2 (Ambient); and 69.3, 62.9–76.8 (Motorboat disturbance).

4.3.4 Identifying impacts of motorboat noise at a single lower-disturbance site

4.3.4.1 Sheltering and chasing behaviour

Male *C. zebroides* are known to exhibit aggressive defence of long-term, visually conspicuous territories containing a feeding area and a sheltering hole (Hert 1992, 1995; Gerlai 2007). Territories were identified by snorkelers along a 400 m stretch of coast, 10–30 m from the shore, at a depth of 2–6 m. Sheltering behaviour and aggressive chasing behaviour were quantified by video analysis using underwater cameras (HERO 4, definition 720p; GoPro, USA). All observed aggressive behaviours occurred within 2 m of the sheltering hole.

An independent-measures experimental design was used, so each fish received one of two sound treatments; sound treatments were alternated between consecutive trials. For each trial, a loudspeaker was suspended 1 m off the lake bed, facing the sheltering hole at a distance of 3 m, on the side of the territory directly opposite to the shore. After a 5-min acclimation period following territory identification and experimental set-up, fish were exposed to 5 min of a 'pre-treatment' ambient-sound playback track. The pre-treatment period was followed immediately by 5 min of a 'sound-treatment' track comprising playback of either a different ambient-sound recording or a motorboat-noise recording. No two territories were closer than 3 m apart, and ambient-sound and motorboat-noise treatments were performed randomly across space within the area, reducing the potential for confounding natural spatial variation.

Videos were analysed by an observer (T.A.C.G.) who was blind to the sound treatment. Sheltering behaviour (defined as the entire body of the fish within the sheltering place) was quantified as a percentage of total time within the camera's field of view. Aggressive chases (defined as sudden increases in speed with simultaneous changes of direction, aimed at fish of other species within the territory) were counted and divided by the total non-sheltering time within the field of view. Periods where fish were outside the field of view were not counted; these periods never exceeded 10 s individually or 10% of the total video time cumulatively. On the one occasion (from 40) where multiple exits from the field of view caused ambiguity about the identity of the focal fish, the video was removed from further analyses. Territory intrusions by conspecific males were never witnessed in any of the videos. The objectivity of both behavioural variables was confirmed by two, independent, experimentally blind observers reporting results that did not significantly differ from each other for the same subset of 10 videos (Sheltering time: Wilcoxon signed-rank tests, $V = 102$, p

= 0.24; Spearman's rank correlation coefficient = 0.998. Aggressive chasing rate: $V = 80$, $p = 0.55$; Spearman's rank correlation coefficient = 0.974).

To assess the impact of motorboat noise, the mean change between pre-treatment and sound-treatment periods in both behavioural variables was compared between sound treatments. This change-based method controlled for inter-individual differences, giving greater statistical power to detect noise-driven patterns in behavioural variables against a background of natural variation. One territory (of 40) where no sheltering or chasing was observed during both pre-treatment and sound-treatment periods was removed from further analyses.

4.3.4.2 Oxygen-consumption rate

During all experimental trials, fish were randomly allocated to different sound treatments to avoid selection bias, and the order of playback tracks or motorboats used was defined randomly within counterbalanced blocks. Treatment order was always alternated to avoid confounding effects of time of day or fish holding-time, and different motorboats and tracks were used in approximately equal numbers of trials.

At the start of each day, male *C. zebroides* were captured at the first lower-disturbance site by snorkelers using a 10 x 1 m barrier net in 2–5 m depth, within a 200 m stretch of coastline. Captured individuals were held, during transport and for storage before testing, in an opaque barrel containing 20 L of regularly flushed lake water. The testing location was within 500 m of capture, transport was by paddling, and the holding barrel was kept on the shore to ensure acoustic isolation from the experimental trials. Fish were released unharmed to their natal sites at the end of each day, and capture was always over 50 m away from capture sites on previous days; due to the high site-fidelity of territorial Lake Malaŵi cichlids (Hert 1992), this avoided the chance of re-testing fish on consecutive days.

Oxygen-consumption rate is an emerging physiological tool for understanding likely impacts of anthropogenic pollutants on ecosystems (Illing and Rummer 2017). The effect of motorboat noise on the oxygen-consumption rate of *C. zebroides* was tested *in situ* using an independent-measures experimental design. Oxygen-consumption rates were compared between fish exposed to either ambient conditions, motorboat disturbance, or their playback equivalents. The complementary use

of real motorboats and loudspeaker playback allowed both acoustic validity and isolation of motorboat noise as a stressor independent of visual cues and wake effects.

For a trial, fish were placed into sections of open PVC piping (9.2 x 3.8 cm) with mesh netting over each end to restrict movement, inside sealed polyethylene terephthalate (PET) containers (13.7 x 6.2 cm; 412 ml volume) suspended from a submerged stand 1.5–3 m below the surface of the lake. Acoustic transparency was predicted to be high due to similar acoustic impedance values for water (1.5 MRayls) and PET (1.76 MRayls). Following a 5-min acclimation period, containers were sealed underwater in the lake and the sound treatment started; trials lasted for 30 min, with four fish run in parallel during each trial. Sealed containers were opaque, eliminating visual cues associated with exposure to motorboat disturbance. At the end of a trial, containers were brought to the surface and fish were removed. All capture, handling and measurement methods were identical for noise-exposed and control fish, facilitating valid comparisons of relative differences in oxygen-consumption rate.

Water temperature and dissolved oxygen content in containers was measured at the start and end of the trial (Dissolved Oxygen and Temperature Meter HI 9164, Hanna Instruments Inc., Woonsocket, USA). Fish length (mean ± SE: 8.51 ± 0.04 cm) and mass (9.24 ± 0.15 g) were recorded immediately after trials. There was no significant difference in either length (one-way ANOVA: $F_{3,70}$ = 0.38, p = 0.77) or mass (Kruskal-Wallis test: H_3 = 0.56, p = 0.91) of fish allocated to different sound treatments. Oxygen-content and mass data were used to calculate oxygen-consumption rates of fish over the trial period (mg $O_2/g_{fish}/h$). To assess the impact of motorboat noise, sound treatment (ambient sound or motorboat noise) and sound source (real sound or loudspeaker playback), and their interaction, were included as predictor variables in a two-way ANOVA.

4.3.5 Testing for effects of motorboat-disturbance history through multi-site comparisons

Having established qualitatively equivalent responses to motorboat disturbance and motorboat-noise playback (see Results), motorboat disturbance was used exclusively for the multi-site comparisons to achieve acoustic validity. Physiological assays were conducted at three additional lower-disturbance sites to investigate whether the oxygen-consumption response detected at the initial lower-disturbance site was consistently found. Assays were then conducted at four higher-disturbance sites to test whether the same response was apparent. Experimental protocols followed those for the initial lower-disturbance site. There was no significant difference in trial temperature

between treatments in either the lower-disturbance (n_{amb} = 71, n_{boat} = 70, t = -0.74, p = 0.46) or higher-disturbance (two-sample t-test: t = -0.42, n_{amb} = 72, n_{boat} = 69, p = 0.67) sites. There was no significant difference in either mass (Mann-Whitney U-tests, lower-disturbance W = 2663, n_{amb} = 71, n_{boat} = 70, p = 0.46; higher-disturbance: W = 2401, n_{amb} = 72, n_{boat} = 69, p = 0.73) or length (two-sample t-tests, lower-disturbance: t = 0.76, n_{amb} = 70, n_{boat} = 65, p = 0.45; higher-disturbance: t = -0.33, n_{amb} = 68, n_{boat} = 64, p = 0.74) of fish allocated to different sound treatments at the lower- and higher-disturbance sites.

Linear mixed models (LMMs), to control for the testing of multiple fish from the same sites, were used to analyse datasets from lower-disturbance and higher-disturbance sites. In both LMMs, sound treatment (ambient or motorboat) was used as a fixed term, site ID (1–4) was included as a random term, and significant effects of sound treatment were confirmed by comparisons with a null model. Visual examination of residual plots never revealed any obvious deviations from homoscedasticity or normality.

4.4 RESULTS

At the initial lower-disturbance site, sound treatment did not significantly affect the change in sheltering time from pre-treatment to sound-treatment period (Mann-Whitney U test: W = 171, n_{amb} = 20, n_{boat} = 19, p = 0.60; Fig. 4.3a). However, the change in aggressive chasing behaviour following the pre-treatment ambient playback period was significantly higher when fish were exposed to motorboat-noise playback compared to ambient-sound playback (W = 100, $n_{amb,\ boat}$ = 19, p = 0.02; Fig. 4.3b). With regards to oxygen-consumption rate, there was a significant effect of sound treatment: fish exposed to motorboat noise exhibited higher oxygen-consumption rates than those exposed to ambient sound (two-way ANOVA: $F_{1,72}$ = 8.42, p = 0.005; Fig. 4.4). However, there was no significant effect of sound source (real sound vs loudspeaker playback) ($F_{1,72}$ = 1.17, p = 0.28), and no significant interaction between sound treatment and sound source ($F_{1,72}$ = 0.80, p = 0.37).

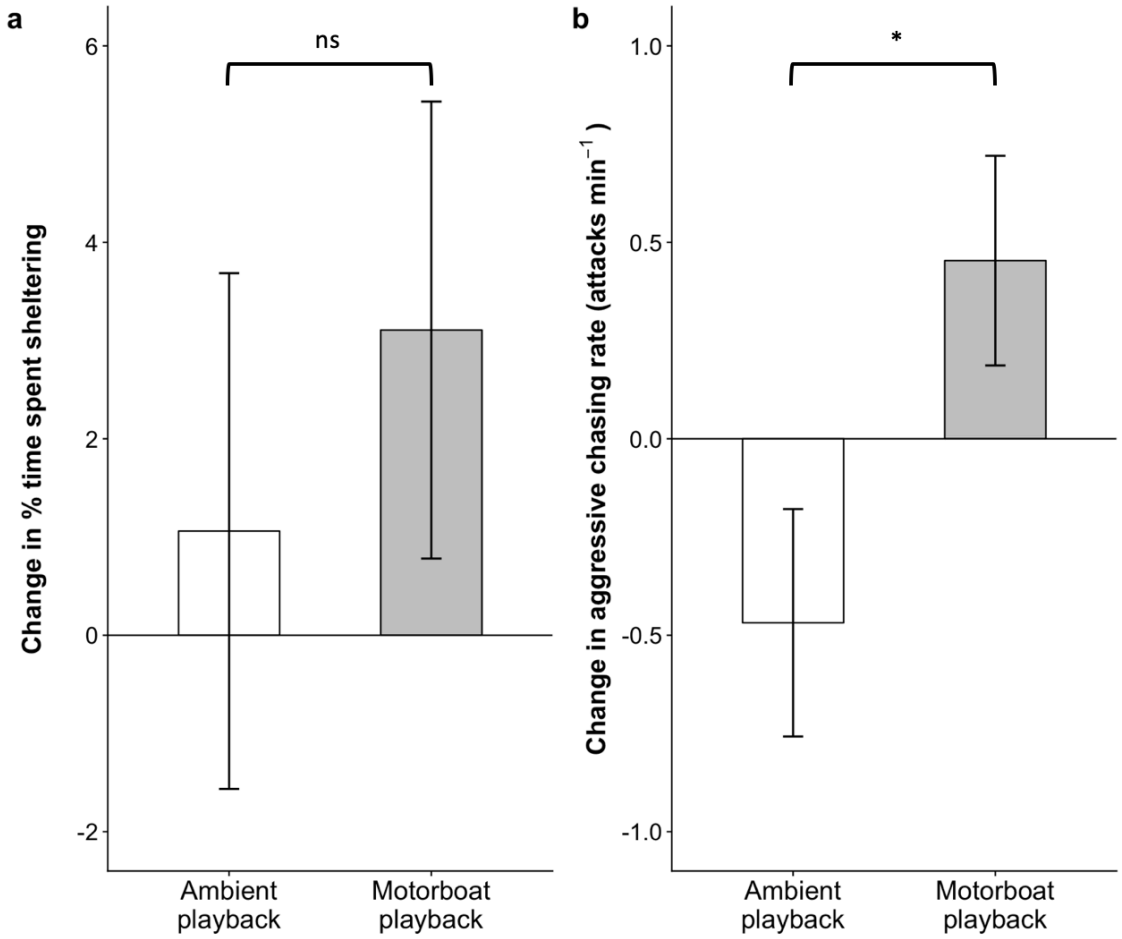

Figure 4.3. Mean ± SE change in (a) % time spent sheltering (ambient-sound playback: n = 20; motorboat-noise playback: n = 19) and (b) aggressive chasing rate (n = 19 for each sound treatment) by fish exposed to playback of either ambient-sound or motorboat-noise treatment following a pre-treatment period of ambient-sound playback. * $p < 0.05$; ns denotes no significant difference.

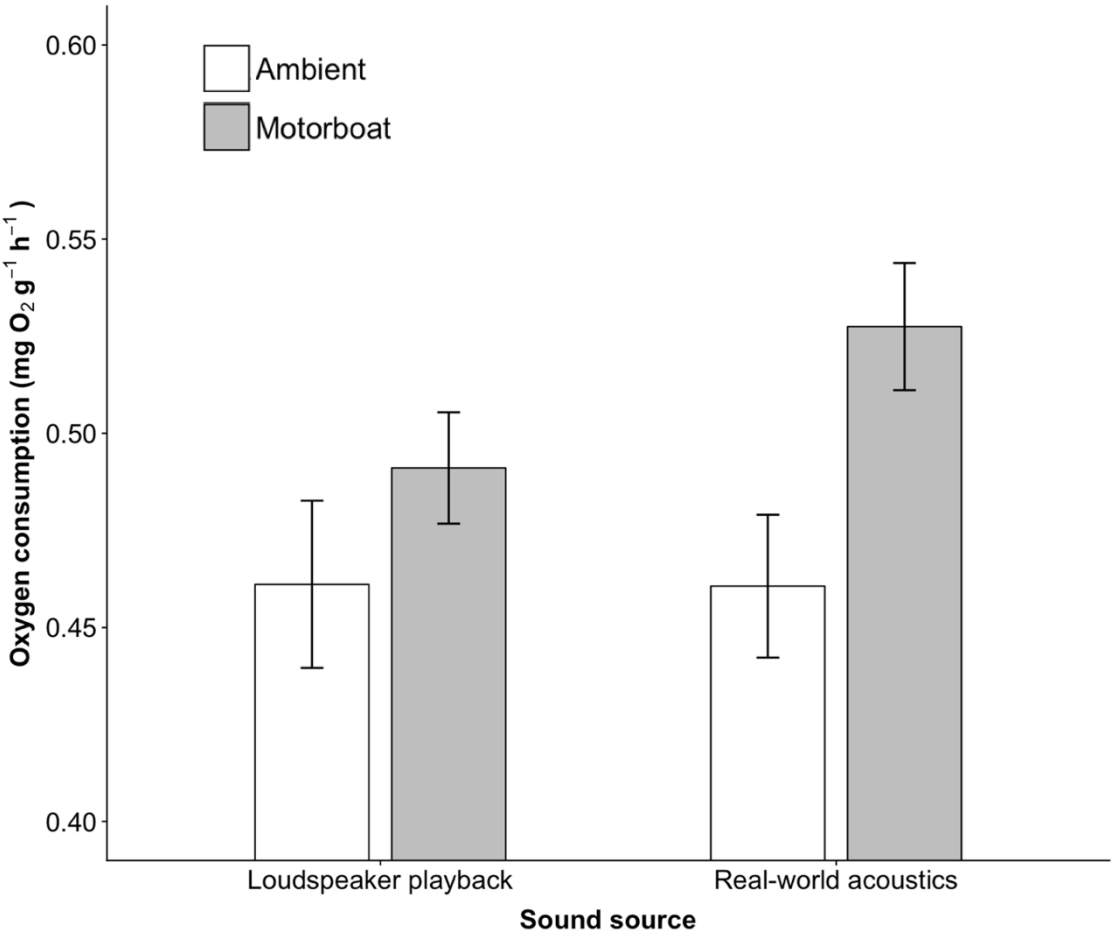

Figure 4.4. Mean ± SE oxygen consumption in *C. zebroides* exposed to playback of ambient sound (n = 19), playback of motorboat noise (n = 20), ambient conditions (n = 18) or motorboat disturbance (n = 19). Sound treatment (ambient sound or motorboat noise) had a significant effect (two-way ANOVA: $F_{1,72} = 8.42$, p = 0.005), but sound source (real sound or loudspeaker playback) ($F_{1,72} = 1.17$, p = 0.28) and its interaction with sound treatment ($F_{1,72} = 0.80$, p = 0.37) did not.

The significant increase in oxygen-consumption rate in response to motorboat disturbance found at the initial lower-disturbance site was replicated when considering all four lower-disturbance sites (LMM: $\chi^2 = 9.239$, df = 1, p = 0.002, Intercept (ambient conditions) ± SE = 0.481 ± 0.008, Unstandardised effect size from model output ± SE = 0.036 ± 0.012; Fig. 4.5a). However, there was no significant effect of motorboat disturbance on oxygen-consumption rate at the four higher-disturbance sites ($\chi^2 = 0.786$, df = 1, p = 0.375, Intercept (ambient conditions) ± SE = 0.480 ± 0.010, Unstandardised effect size from model output ± SE = 0.012 ± 0.014; Fig. 4.5b). The variance associated with the random 'Site ID' term was less than 0.001 in both cases (Variance ± SD: Lower-disturbance <0.001 ± <0.001; Higher-disturbance <0.001 ± 0.008).

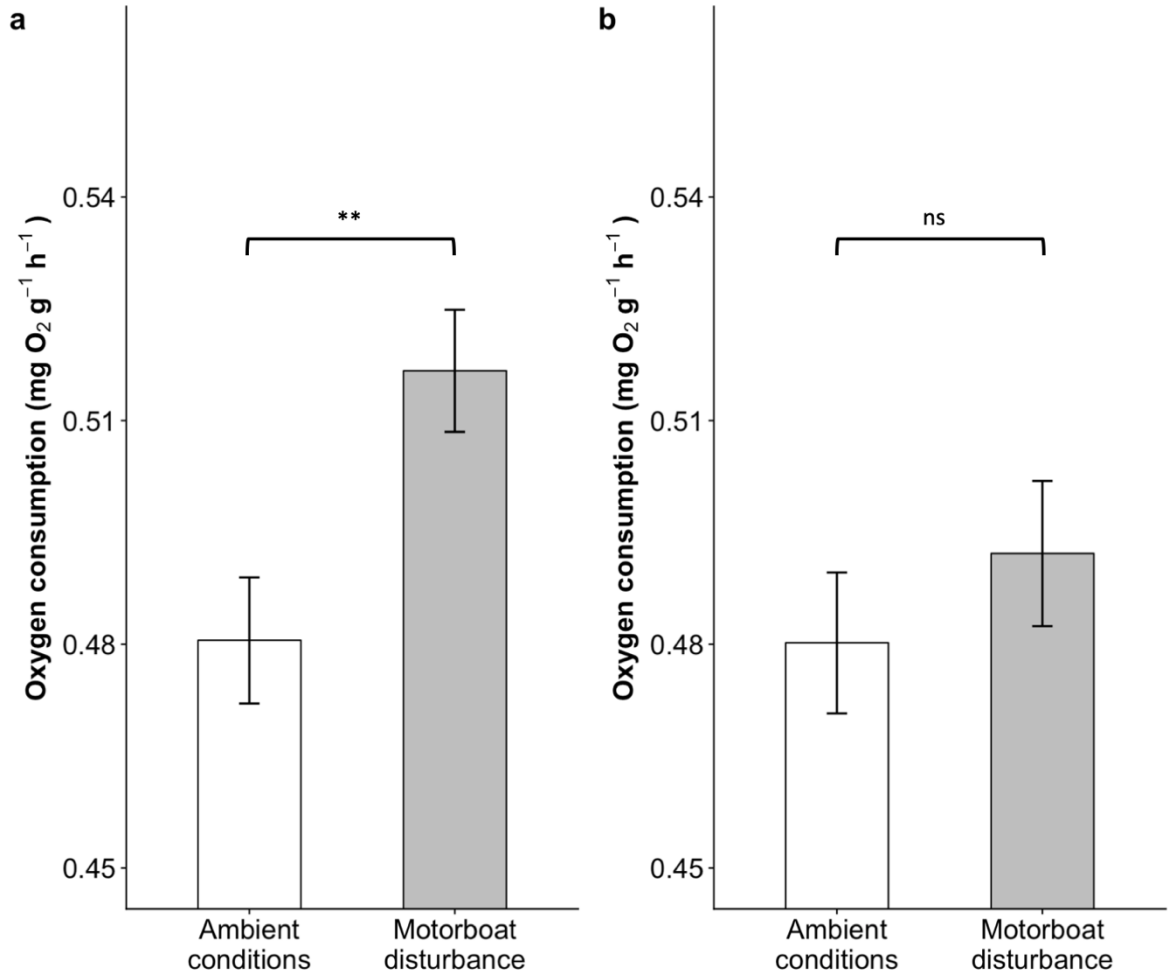

Figure 4.5. Mean ± SE oxygen consumption in *C. zebroides* exposed to ambient conditions or motorboat disturbance in areas with (a) lower (ambient: n = 72; motorboats: n = 69) or (b) higher (ambient: n = 71; motorboats: n = 70) current and historic levels of motorboat activity. ** $p < 0.01$; ns denotes no significant difference.

4.5 DISCUSSION

Using field-based experiments, we identified negative effects of motorboat-noise playback on behavioural and physiological responses of wild endemic cichlids in Lake Malaŵi. We also found an equivalent physiological response to motorboat-noise playback and the driving of real motorboats. Moreover, using the physiological assay, we demonstrated that responses to motorboat noise were lower in areas with higher levels of motorboat disturbance compared to those with lower disturbance levels. This represents novel *in situ* evidence to add to a small but increasing body of work examining intraspecific variation in organismal responses to noise (Naguib et al. 2013; Purser et al. 2016; Radford et al. 2016b).

Aggressive chasing behaviour increased when fish were exposed to motorboat noise, despite equivalent decreases in the fish exposed only to ambient sound. Decreases in aggression in fish exposed only to ambient sound were likely due to continued acclimation after disturbance during experimental set-up. The reverse effect during motorboat-noise playback has been shown in another cichlid species (*Neolamprologus pulcher*) (Bruintjes and Radford 2013), and may be driven by a number of different reasons. First, acoustic signals are important in mediating agonistic interactions in other cichlid species, and masking of such signals used in territory defence may alter contest outcomes with conspecifics, as has been shown in the red-mouthed goby (*Gobius cruentatus*) (Schwarz 1974; Sebastianutto et al. 2011). Second, individuals may be distracted by noise, directly affecting the detection and processing of important cues, including those from other sensory modalities (Chan et al. 2010; Chan and Blumstein 2011). This may mean that focal fish miss cues involved in alleviating interactions before they escalate to chases. Finally, noise may act as a stressor that results in the release of corticosteroid hormones (e.g. cortisol) (Barton 2002); these steroid hormones have been shown to influence agonistic interactions of territorial fishes (Winberg and Nilsson 1993; Ros et al. 2014). For example, cichlid fish (*Aequidens pulcher*) treated with cortisol were shown to be more aggressive to a model intruder (Munro and Pitcher 1985). However, it is important to note that the observed territorial changes might be driven by behavioural changes in territory defenders, intruders, or both. Increases in aggressive behaviour are of likely ecological importance; aggressive defence of reproductive territories is common amongst males of many Lake Malaŵi cichlid species (Hert 1992; Konings 2001). Further, territoriality in fish can be an important driver of wider ecosystem dynamics, affecting variables such as benthic community structure and ecosystem productivity (Klumpp et al. 1987; Ceccarelli 2007; Gordon et al. 2015).

The use of a rapid-assessment physiological assay revealed an increase in oxygen-consumption rate in fish exposed to motorboat noise, as has been shown in other fish species (Simpson et al. 2015, 2016a) and other taxa (Wale et al. 2013). Oxygen uptake is used as a secondary measure of stress, due to the link between release of corticosteroid hormones and oxygen uptake (Brown et al. 1982; Nadler et al. 2016). The observed increase in oxygen-consumption rate of noise-exposed fish links directly with, and may underpin, the plausible mechanisms affecting behaviour described above.

In this study we found an equivalent response to both loudspeaker-playback of motorboat noise and real motorboats. Each method has advantages and limitations in terms of acoustic and behavioural validity, with their complementary use providing the greatest insight (Slabbekoorn

2016). The use of playback isolates noise as the stressor, free from confounding visual and wake effects, whilst real motorboats provide real-world validity. Unfortunately, the complementary use of both methods in research studies is rarely used (see Simpson *et al.* 2016a for an exception). Addressing the impacts of noise in field-based experiments not only enables the use of real-noise sources, but provides an acoustic signal in an open soundscape and avoids the complex acoustics generated in tank environments (Okumura et al. 2002). Wild behavioural observations in natural settings provide ecological validity in allowing the focal individual to display its full behavioural repertoire, which may be absent in constrained animals (Radford et al. 2014). However, field experiments inherently suffer from extraneous variables and are more difficult to control. To date, a large number of noise studies have primarily been conducted in captive conditions (Voellmy et al. 2014a; Radford et al. 2016b), with a few notable exceptions of those using real-noise sources in natural settings (Holmes et al. 2017; Nedelec et al. 2017b). Aiming to incorporate both elements into studies represents the gold standard for researching impacts of noise pollution.

The observed difference in response to short-term motorboat noise at lower- and higher-disturbance sites is unlikely due to ecological differences in the sites, as they were matched by depth, distance to shore, benthic substrate and water temperature. Instead, the differences in disturbance history offers the most plausible explanation. In contrast to previous laboratory-based extended exposures of fishes to noise (Radford et al. 2016b), the lower response of fish at natural higher-disturbance sites could represent either acclimation within a generation (e.g. increased tolerance through shifts in hearing sensitivity thresholds, or a declining response from learning that the stimulus does not have any detrimental consequences (Scholik and Yan 2002; Bejder et al. 2009) and/or adaptation through selection over multiple generations (Møller 2008; Nemeth et al. 2013). Indeed, tolerance may itself be a phenotypic trait subject to plasticity; for example, organisms with a higher tolerance of noise may have a selective advantage in higher-disturbance areas through increased opportunities for foraging and mating (Lowry et al. 2011; Nemeth et al. 2013). Future work could use extended field-based manipulations of motorboat exposure within a generation to isolate disturbance history from any unmeasured and potentially confounding variables. This would facilitate further understanding of both the mechanistic drivers and the timescale over which such changes in tolerance develop (Bejder et al. 2009).

Considering intraspecific variation in response to noise is vital in order to gain a full understanding, and avoid an over-simplified misrepresentation, of the impacts of anthropogenic noise on individuals, populations, communities and ecosystems. By investigating responses based on

varying intrinsic characteristics (e.g. body size, personality, sex) and extrinsic factors (e.g. environmental context, prior experience) we can improve management practices, monitoring assessments and mitigation measures. For example, female European eels (*Anguilla anguilla*) decline in physiological condition when investing in egg development; eels in poorer physiological condition display reduced anti-predator vigilance and may be more at risk when exposed to noise, suggesting mitigation strategies should consider both the timing of noise-generating activities and the need for a recovery period post-spawning (Purser et al. 2016). Further, intraspecific variation in responses to other stressors has been shown to influence population dynamics, community structure and ecosystem functioning (Post et al. 2008; Rudman et al. 2015; Charette and Derry 2016; Des Roches et al. 2017). Considering only 'mean' responses to anthropogenic noise may underappreciate the likely consequences of the disturbance.

Our study provides evidence from wild fish that physiological responses to motorboats can be affected by existing variation in disturbance history. Such intraspecific variation has implications for understanding and mitigating effects of noise on wildlife; for example, ecosystem impact assessments carried out in historically disturbed areas may represent underestimates of the threats posed to wider populations by novel sources of noise pollution. Despite individuals from these disturbed areas showing a reduced sensitivity to noise, more subtle detrimental consequences may be present; there may still be declines in growth and fitness consequences for those individuals (Slabbekoorn et al. 2010). Given the results here, we advocate more work on noise pollution that moves beyond consideration of whether there is an impact to investigations of what causes variation in responses.

Chapter 5 – Chronic motorboat disturbance alters coral reef fish communities

Contributions to the work

H.R.H., E.W. (University of Exeter Masters Student; Masters project on anthropogenic noise with in-field supervision by H.R.H and acted as field assistant) and supervisors S.D.S. and A.N.R. conceived the research; H.R.H., E.W., R.B. (CRIOBE Researcher; provided valuable local logistical and in-field advice), S.C.M. (Lecturer in Coral Reef Environmental Change; provided valuable local logistical and in-field advice), S.D.S. and A.N.R. designed the experiments; H.R.H. and E.W. conducted the fieldwork. H.R.H. analysed the single-species behavioural experiments; E.W. analysed the community data with the help of H.R.H. H.R.H. and E.W. interpreted the results, advised by S.D.S. and A.N.R.; H.R.H. wrote the chapter, with comments provided by S.D.S. and A.N.R.

This chapter is being prepared for publication as:
Harding HR, Weschke E, Beldade R, Mills SC, Simpson SD, Radford AN. Chronic motorboat disturbance alters coral reef fish communities. Nature Ecology and Evolution.

5.1 ABSTRACT

Anthropogenic noise is a pollutant of global concern. Motorboat noise is pervasive in many coastal regions, with its prevalence increasing worldwide. Previous work has shown motorboat noise to affect the physiology (e.g. increases in oxygen-consumption rates and heart rates, changes in growth rates) and behaviour (e.g. orientation, foraging, predator avoidance) of fishes. However, much of this research has focussed on the responses of single species to acute noise exposures. Wild fishes likely experience repeated exposures of anthropogenic noise over chronic periods of time, which may alter any observed responses. Additionally, to understand fully the potential impacts of anthropogenic noise on ecosystems, the effects on entire communities need to be determined. Here, we first used existing variation in motorboat traffic to demonstrate differences in the abundances of a number of coral reef fish species between high- and low-disturbance sites, which results in different community structures. We then conducted a month-long experiment, manipulating the level of chronic motorboat disturbance in natural areas with low previous acoustic history. In replicating a number of the findings of the observational study, in terms of species abundances, we provide the first field experimental evidence that motorboat disturbance levels can have effects on fish communities. Further, we assessed whether the responses to noise exposure of a dominant member of the reef fish community (*Stegastes nigricans*) changed over the same chronic motorboat-disturbance period. We found no significant effects of short-term exposure to noise, nor altered responses following the manipulated disturbance period. The varied responses shown amongst different members of the coral reef fish community suggest that we should not just consider species responses to noise in isolation but must consider the chronic effects of anthropogenic noise on entire communities.

5.2 INTRODUCTION

Anthropogenic noise is recognised as a pollutant of global concern in both terrestrial and aquatic environments (Hildebrand 2005; Buxton et al. 2017). Many noise-generating activities occur in coastal regions (Davenport and Davenport 2006), with human population growth, infrastructure development and increased transportation, fishing and tourism leading to ever-greater levels of motorboat traffic in particular (Whitfield and Becker 2014). Coral reefs form a small yet essential component of tropical coastal waters: despite making up less than 1.2% of the world's continental shelf area, they are home to 25% of the world's marine fishes (Spalding et al. 2001). Reef fishes often live in close proximity to motorboat noise, therefore, and recent research has demonstrated

that noise from this source can have a range of physiological, developmental and behavioural impacts on individual fish (reviews: Slabbekoorn et al. 2010; Kunc et al. 2016; Cox et al. 2018). However, the majority of studies have only assessed the short-term effects of motorboat noise on a single species. In the wild, fishes are subject to repeated exposures to motorboats, and there are likely to be interspecific differences in how they are affected; as such, how individuals and entire communities are affected by chronic motorboat exposure needs experimental consideration.

Chronic exposure to noise may result in changed responses by individuals as a result of hearing threshold shifts, altered tolerance, habituation or sensitisation; at the population level, responses may change due to evasive emigration of more sensitive individuals (Scholik and Yan 2002; Bejder et al. 2009; Radford et al. 2016b). Previous studies have shown that acoustic history can affect responses to subsequent short-term noise exposure (Nedelec et al. 2016b; Radford et al. 2016b; Harding et al. 2018). This small body of research has commonly identified that pre-exposed fishes have lessened physiological and behavioural responses to additional noise, including from motorboats. However, this previous work either used captive fish or defined pre-exposure levels using historic natural variation in motorboat traffic. Experiments are needed that test free-swimming wild fish before and after a chronic period of manipulated motorboat disturbance to assess the capacity for behavioural plasticity. The ability of organisms to adjust their behaviour is often the first line of defence when they are confronted with novel stressors (Wong and Candolin 2015); exploring whether individuals are able to adjust their behaviour to noise with continued exposure will develop our understanding of population responses and inform mitigation strategies.

Interspecific variation in responses to noise is expected in fish as they possess a greater diversity of sound-production and detection mechanisms, with associated variation in sensitivity, than any other vertebrate group (Ladich 2000; Popper and Fay 2011). As such, differences in hearing sensitivities and noise tolerances could greatly influence the likely winners and losers in a community exposed to anthropogenic noise. There is some limited evidence from captive studies of different responses by different fish species to the same noise playbacks (Voellmy et al. 2014a,b). Moreover, there are some hints of interspecific variation from recent work showing alterations in predator–prey dynamics and mutualistic interactions when pairs of species are exposed to noise (Simpson et al. 2016a; Nedelec et al. 2017b). Understanding potential changes in such interactions is important for determining how ecological processes are affected, but is not sufficient to predict impacts on entire communities. While there have been some assessments of community-wide impacts of anthropogenic noise in terrestrial systems (Francis et al. 2009; Herrera-Montes and Aide

2011), we know of only one related study in a marine system: in an observational study, Correa *et al.* (2019) used passive acoustics to show that there was reduced fish call complexity in the presence of motorboat disturbance. However, experimental tests of marine communities exposed to chronic anthropogenic noise are now needed to understand the potential effects on species distributions. Determining how communities will be affected in an increasingly noisy world is vital in order to predict how ecosystem function and stability may change in the future.

The current study aimed to investigate, for the first time, the ecological implications of anthropogenic noise on a coral reef fish community, and to test for changes in behavioural response in one of the most dominant members of the community (*Stegastes nigricans*) following chronic noise disturbance. We first used existing spatial variation in motorboat traffic across coral reefs to determine whether fish community assemblages differ in relation to disturbance levels. This observational portion of the study explored whether long-term motorboat disturbance: (1) alters overall fish abundance, species richness and diversity; and (2) generates species abundance differences between high- and low-disturbance areas. To isolate the importance of motorboat disturbance, we conducted a month-long experimental manipulation of natural sites with minimal previous disturbance history. This allowed us to assess whether: (1) behavioural responses of *S. nigricans* change after a chronic regime of motorboat disturbance; and (2) if similar differences in community assemblages are seen between sites of high and low experimental disturbance as those seen in sites that differ in historical disturbance levels.

5.3 METHODS

5.3.1 Study system and sites

All observational and experimental work was conducted during October–November 2017 in the lagoon surrounding the Pacific Island of Mo'orea, French Polynesia. Observational data were collected on fish communities on the north fringing reef of Mo'orea along two boat channels that have been active for over 30 years (Fig. 5.1a–b). The Matautia (mean ± SE width: 17 ± 3 m; length: 1.23 km) and Vaipahu (width: 17 ± 1 m; length: 1.61 km) boat channels allow the safe passage for tourism vessels, pleasure craft and artisanal fishing boats through continuous sections of reef habitat in the lagoon. As a result, the channels receive regular motorboat traffic (Matautia: 17.7 $\pm$ 1.9 (mean $\pm$ SE) boats per hour, 10 h of observation across four days; Vaipahu: 5.7 $\pm$ 0.9 boats per hour, 15.5 h of observation across 9 days). Community assemblages were considered at two

distances perpendicular to the boat channels (see below), allowing a comparison of high- and low-disturbance sites (Fig. 5.1a–b).

Sites for experimental manipulation of motorboat disturbance were situated on the north-western side of the island (Fig. 5.1c), 170–340 m from the nearest boating channel (5.5 $\pm$ 0.9 boats per hour were seen operating in the distant channel, 77 h of observation across 25 days; only 1.5% of boat passes were closer to the study sites than the distant channel). The spatial separation of individual coral bommies (isolated sections of reef) in this area facilitated experimental motorboat driving. As well as an assessment of fish community assemblages, behavioural responses of the dusky farmerfish (*Stegastes nigricans*) were determined in relation to motorboat exposure. *S. nigricans* is a territorial damselfish that, through competitive exclusion, maintains algal farms by non-selective weeding of indigestible algal species (Hata and Kato 2002; Hata and Umezawa 2011). Individual territories are often adjacent to each other, resulting in the formation of large colonies (Karino and Nakazono 1993). The territorial behaviour and associated high site-fidelity (Lecchini and Galzin 2005; Lo-Yat et al. 2005; Blanchette et al. 2019) makes *S. nigricans* a useful study species for repeated observations of the same individuals.

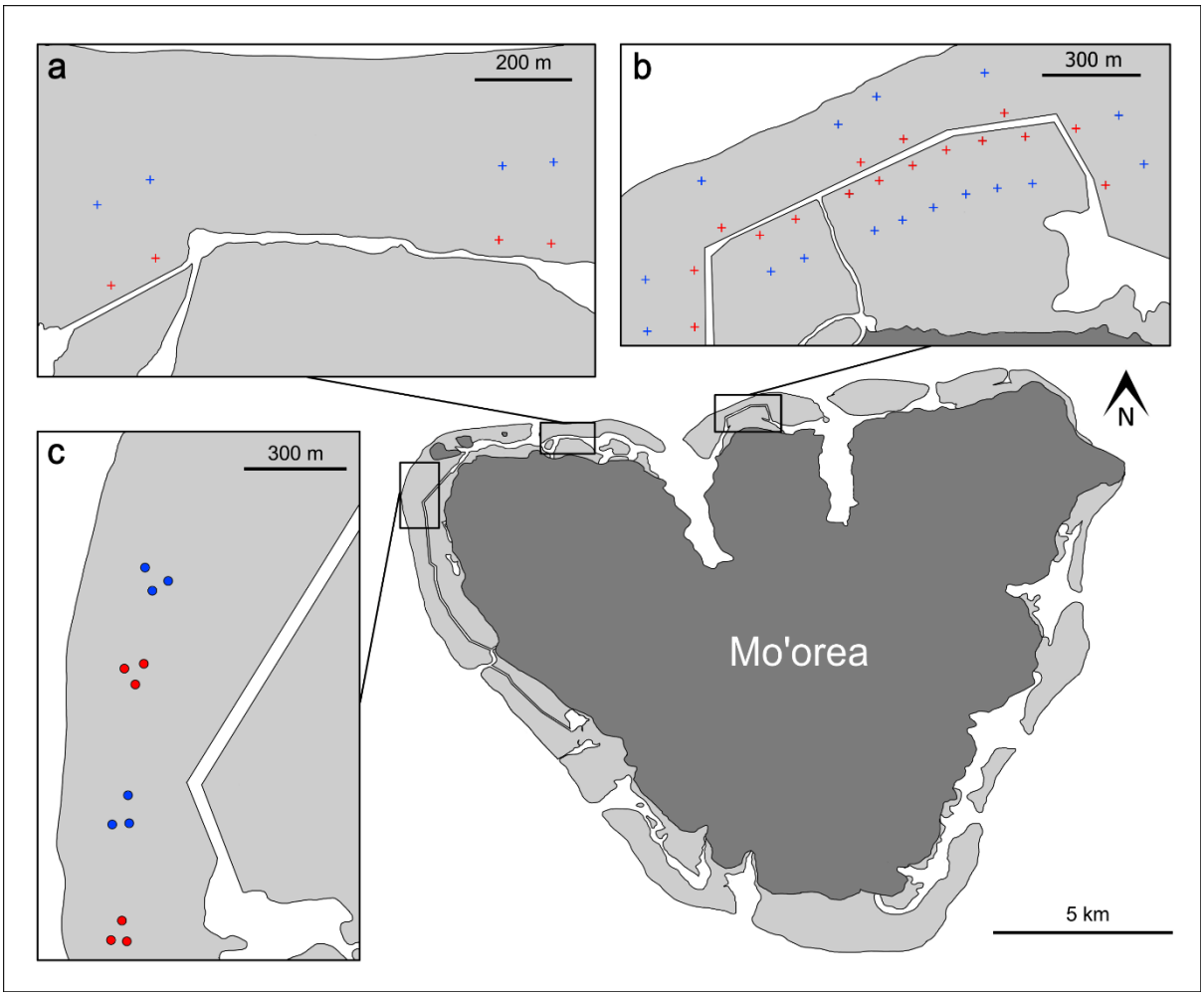

Figure 5.1. Map of Mo'orea (dark grey), its surrounding reef (light grey) and boat channels (white). The observational fish-community study sites, denoted with red (high disturbance) and blue (low disturbance) crosses and paired within sampling lines, were situated at the northern (a) Matautia and (b) Vaipahu boat channels. Experimental-manipulation study sites, denoted with red (chronic motorboat disturbance) and blue (chronic ambient) circles, were situated on the (c) north-western reef away from any boat channels.

5.3.2 Fish community differences in relation to existing variation in motorboat disturbance

Reef sites within 20–40 m of the boat channels were characterised as 'high disturbance'; the minimum distance of 20 m from the channel reduced the potential influence of other channel-associated factors on fish assemblages, including edge effects (Sambrook et al. 2016) and the wake and visual stimulus of passing boats. Reef sites between 100 and 140 m from the boat channels were characterised as 'low disturbance'. High- and low-disturbance sites were paired within 'sampling lines', which extended perpendicular from the channel, to account for potential larger-scale

variation in fish assemblages along the >1 km boat channels. At each high- and low-disturbance site, 50 m survey transects were sampled parallel to the boat channel; transects at neighbouring sampling lines were separated by a minimum of 30 m. Overall, 20 sampling lines (each with a high- and low-disturbance site) were established along the two channels, resulting in 40 transects in total. Constraints in habitat type (large regions of sand and extremely shallow water that would have heavily driven variation in fish assemblages) and the minimum separation distance between survey sites meant that the Matautia channel was restricted to four sampling lines; there were 16 sampling lines along the Vaipahu channel.

Benthic surveys were undertaken to assess substrate composition along the transects. Benthic video recordings were made for each 50 m transect by one surveyor (H.R.H.) swimming 1 m above the reef and filming with a GoPro Hero 4 from a standardised mounted-camera position. The point-intercept method (Ohlhorst et al. 1988) was then used on these video recordings to quantify the benthic composition. Using a consistent midpoint of the video panel (VLC Media Player), the substrate type at 1 m intervals along the transects was categorised as one of the following: live coral (soft and hard), dead coral (algal symbionts visibly absent), turfing/macroalgae, sand, rubble, bedrock, anemone or human material. Benthic composition did not vary significantly between high- and low-disturbance sites (PERMANOVA: Pseudo-F = 0.083, p = 0.953; Fig. C.1, Appendix C). Depth was measured at 5 x 10 m intervals along each transect using a dive computer (Zoop, Suunto Oy, Vantaa, Finland). High-disturbance sites were marginally deeper than low-disturbance sites (mean ± SE difference: 16 ± 0.1 cm; paired t-test: t = 3.12, df = 19, p = 0.006). However, this difference in depth was well within the tidal range (0.3 m from MHWS to MLWS) and similar to the resolution of the dive computer (± 1% accuracy; 0.1 m resolution), and thus is unlikely to influence fish assemblages.

Non-cryptic fish were visually surveyed along transects by snorkelers and classified to species. The first of two surveyors (E.W.) documented transient mobile fish species within a 4 m-wide belt. After a period of 3 min, allowing fish to resume typical behaviour, the second surveyor (H.R.H.) documented site-attached fishes within a 2 m-wide belt. Surveys were conducted between 09:00 and 16:00 to avoid crepuscular periods of high temporal variation in fish assemblages (Mallet et al. 2016). Each site was surveyed three times, with a minimum of 24 h between repeats.

5.3.3 Experimental manipulation of motorboat disturbance

Single-species behavioural responses were measured, and community assemblages were censused, at 12 sites before the start of an experimental regime of chronic motorboat disturbance or an equivalent control period. The 12 sites were clustered in groups of three (minimum separation distance of 40 m within a cluster), with the four clusters separated by a minimum distance of 170 m, along the back reef within the lagoon (Fig. 5.1c). Clustered grouping of reefs aided boat operations during the experimental manipulation. The disturbance regime consisted of driving motorboats at two of the clusters (hereafter chronic-motorboat sites), with the other two clusters acting as controls and receiving no experimental motorboat disturbance (chronic-ambient sites). The driving regime involved repeated passes 10–50 m from the site for 5 min before moving onto the next site in the cluster. Three 25 hp motorboats were used in a randomised order over the course of the disturbance regime to minimise pseudoreplication. Motorboat exposures were carried out for 15 min at each chronic-motorboat cluster, twice per day for 22 days and once per day for 7 days (depending on weather and boat availability) during the course of one month. Therefore, each chronic-motorboat cluster received 12.25 h total experimental motorboat exposure; each site received ~4 h of close-proximity motorboat disturbance and ~8 h of varying levels of motorboat disturbance from motorboats driving around the other sites in the cluster.

5.3.3.1 Stegastes nigricans *behaviour*

Eighty-eight *S. nigricans* territories on coral bommies were randomly selected across the chronic-motorboat and chronic-ambient sites for short-term trials consisting of exposure to motorboat disturbance or ambient conditions. The adult individuals selected for observation were all of a similar size to reduce the potential for variation of behavioural responses due to differences in age or ontogeny (Letourneur et al. 1997; Harding et al. 2019). Short-term trials were conducted both before and at the end of the chronic-manipulation period. For all short-term trials, a video camera (GoPro Hero 4) mounted on a stand was positioned to capture the behaviour of the focal fish, its sheltering space and the surrounding territory. Once in position, filming started with a 10-min acclimation period, after which baseline behaviours were recorded for 5 min in ambient conditions, and then there was a 5-min treatment period consisting of either motorboat disturbance or ambient conditions (as a control). All trials were carried out between 09:00 and 17:00 to avoid crepuscular behaviour, and the order of short-term motorboat-disturbance and ambient-control trials was

alternated between sites to avoid diurnal biases in behaviour. Once an initial short-term trial was completed, each *S. nigricans* territory was marked with a label and a GPS point. This allowed an easy return following the chronic experimental-manipulation period, when the same short-term disturbance treatment (ambient or motorboat) was repeated at each particular territory.

Each trial video (pre- and post-chronic manipulation) was cut to remove the 10-min acclimation period and thus leave two 5-min videos consisting of the ambient-baseline period and the treatment period (short-term ambient conditions or motorboat disturbance). A similar proportion of videos from each chronic and short-term treatment combination, and from the pre- and post-chronic periods, were randomly allocated into four separate folders, which were analysed sequentially to avoid any bias from changes in scoring over time. All videos were analysed blind to treatment and period, by H.R.H. using event logging software BORIS (Behavioural Observation Research Interactive Software; v.6.2.4; Friard and Gamba 2016). The following behavioural metrics were scored: (1) proportion of time the focal fish spent sheltering (sheltering started when the entire body of the fish entered the sheltering space); (2) number of bites directed at the substrate per minute; (3) occurrences of debris/sediment clearing (Ceccarelli et al. 2001); and 4) occurrences of aggressive chases (a sudden increase in speed and simultaneous directional change directed towards another fish). All videos where the fish was out-of-frame for >50% of the time and where the focal fish was in view for <30 s were removed from further analysis (n = 11, from 176); videos where the focal fish had disappeared from their territory following the chronic-manipulation period were also removed (n = 6, from 88). In each of these cases, the matched trial from the pre- or post-chronic-manipulation period for the same fish was removed from the dataset. This resulted in sample sizes of: n = 18 for chronic ambient, short-term ambient; n = 16 for chronic ambient, short-term motorboat; n = 20 for chronic motorboat, short-term ambient; and n = 17 for chronic motorboat, short-term motorboat. The overall time spent sheltering for each fish was calculated as a percentage of the total video time excluding the amount classified as out-of-frame. The number of bites whilst in frame (excluding sheltering and out-of-frame time) was used to calculate a rate per minute. Debris/sediment-clearing and chasing data were converted into binomial response terms (0 = no occurrence of behaviour, 1 = occurrence of behaviour) for further analysis as they were relatively rare events.

5.3.3.2 Fish community surveys

Fish surveys were carried out at the 12 chronic-manipulation sites using the same methods as for the existing motorboat-disturbance sites. The 50 m transects passed through the immediate vicinity of the *S. nigricans* territories selected for the behavioural experiment. A single survey was conducted at each of the 12 sites before the chronic-manipulation period. Transects were visibly marked to enable easy return and repeat of an equivalent survey 40 days later (post-chronic-manipulation period). Benthic composition was assessed at each site, following the methods described above; benthic composition did not differ significantly between the chronic-motorboat and chronic-ambient sites (PERMANOVA: Pseudo-F = 0.172, p = 0.955, Fig. C.2, Appendix C). The depth ranged from 1–2 m, measured with the same method as above. There was no significant difference in depth between the chronic-motorboat and chronic-ambient sites (independent-samples t-test: t = -0.28, df = 7.17, p = 0.79).

5.3.4 Acoustic stimuli and analysis

To characterise the soundscape experienced by the fish communities at the existing high- and low-disturbance sites, representative examples of passes by motorboats routinely using the channels and of ambient conditions were recorded. Recordings were made in both acoustic-pressure (Fig. 5.2a) and particle-motion (Fig. 5.2b) domains at sites across the two boat channels. To characterise experimental-manipulation acoustic conditions, representative examples of ambient sound and motorboat noise at individual sites, equivalent for both the short-term experimental behavioural trials and the chronic driving regime carried out at each site, were recorded in both acoustic-pressure (Fig. 5.2c) and particle-motion (Fig 5.2d) domains. To ensure that experimental noise from the chronic-motorboat sites was not propagating to the chronic-ambient sites, representative acoustic conditions at adjacent control clusters were recorded whilst driving boats at chronic-motorboat sites; these recordings were in the particle-motion domain only, due to the hydrophone not being available (Fig. 5.2d).

Acoustic pressure was measured using a calibrated omnidirectional hydrophone (HiTech HTI-96-MIN with inbuilt preamplifier, manufacturer-calibrated sensitivity -164.3 dB re 1 V/µPa; frequency range 0.02–30 kHz; High Tech Inc., Gulfport MS) and a digital recorder (PCM-M10, 48 kHz sampling rate, Sony Corporation, Tokyo, Japan). Particle motion was measured using a calibrated triaxial accelerometer (M20-040; sensitivity 0–3 kHz; Geospectrum Technologies, Dartmouth, Canada) and a 4-track recorder (Boss BR-800, 44.1 kHz sampling rate, Roland Corporation, Los Angeles, CA). All recording levels used were calibrated using a 1 kHz pure sine

wave signal of known voltage, measured in-line with an oscilloscope. Artefacts (external noise from equipment knocking) detected in the recordings were removed following manual inspection of spectrograms using Audacity 2.3.0 software (www.audacityteam.org). Due to the close proximity of our hydrophone to fish territories, fish vocalisations formed a large part of the soundscape below 600 Hz; in order to gain an accurate assessment of the contribution of motorboat noise to high- and low-disturbance sites, these vocalisations were manually removed after inspection of spectrograms (Fig. 5.2a–b; see Fig. C.3a–b, Appendix C, for power spectral density plots of recordings before removal of fish vocalisations).

The acoustic-pressure and particle-motion recordings taken at the high- and low-disturbance sites were cut into 20 s clips for each treatment—high-disturbance sites with a boat pass (10 s either side the peak of the pass), high-disturbance ambient conditions, and the equivalents at the low-disturbance sites—and appended together. Recordings of experimental manipulations from boat sites were cut into 5-min clips to reflect exposures during short-term trials; two recordings to test for acoustic transfer of motorboat noise between chronic-motorboat and chronic-ambient clusters were assessed over 3 min.

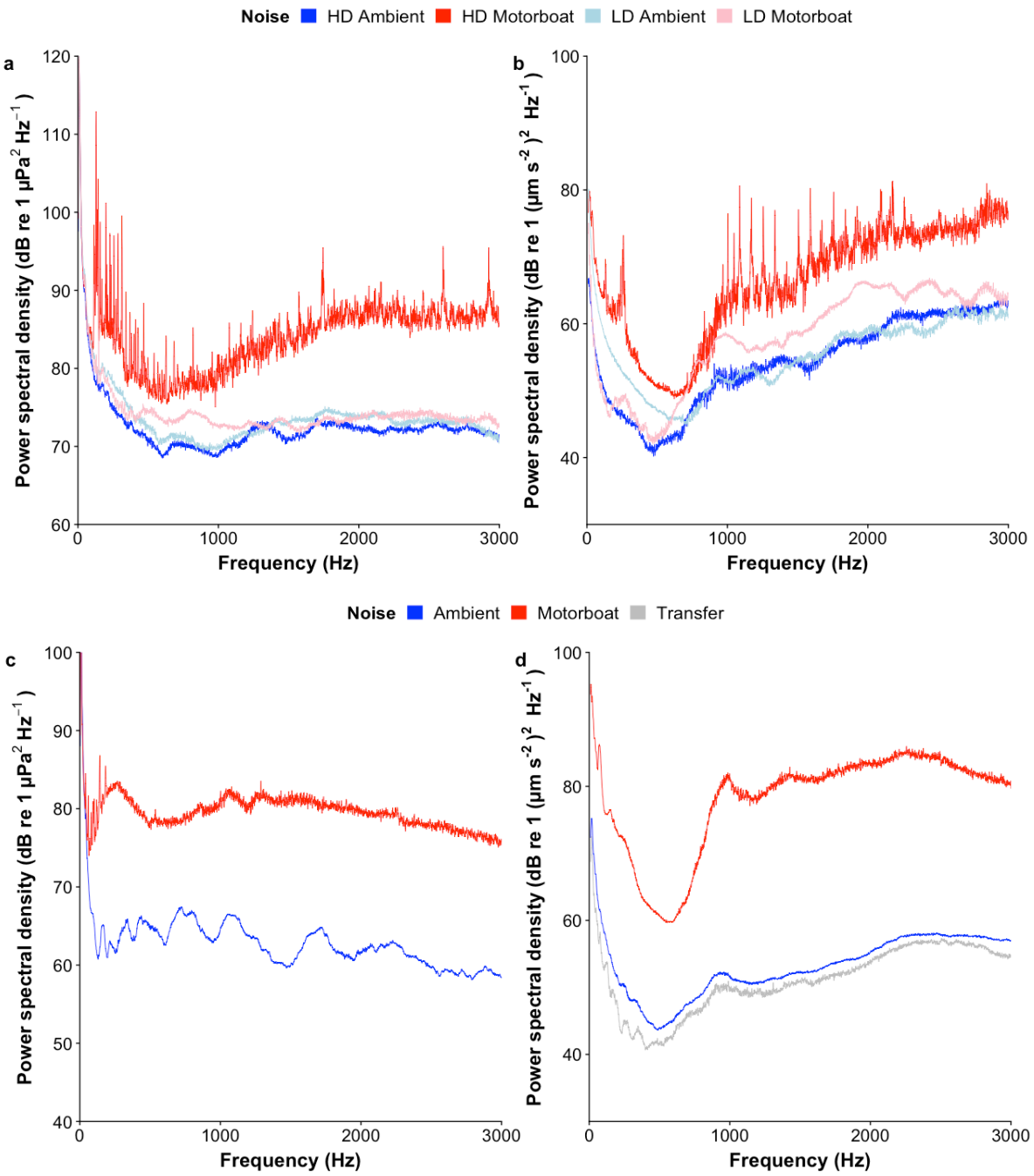

Figure 5.2. Power spectral density plots of sound measurements from the motorboat channels and chronic-manipulation sites. All recordings were analysed using *PaPAM* acoustics analysis package in MATLAB (Nedelec et al. 2016a), with a sampling rate of 44.1 kHz, Hamming window, 50% overlap, window length = sampling rate. HD = High-disturbance sites, LD = Low-disturbance sites. Acoustic pressure at the (a) boat-channel sites ($n_{high\text{-}disturbance}$ = 14; $n_{low\text{-}disturbance}$ = 14) and the (c) chronic-manipulation sites ($n_{ambient}$ = 4; $n_{motorboat}$ = 4), and monoaxial particle motion at the (b) boat-channel sites ($n_{high\text{-}disturbance}$ = 4; $n_{low\text{-}disturbance}$ = 4) and the (d) chronic-manipulation sites ($n_{ambient}$ = 8; $n_{motorboat}$ = 2; $n_{transfer}$ = 2). Lines represent the mean power spectral density for each treatment condition.

5.3.5 Statistical analysis

All multivariate analyses were performed in PRIMER v6 with +PERMANOVA add-on package (Clarke and Warwick 2001). All univariate analyses were performed in R (www.cran.r-project.org, version 3.5.2) using linear mixed models (LMM) or generalised linear mixed models (GLMMs), with the error structure determined using AICc selection (R packages: *lme4*; *MuMIn; DHARMa*). Levels of significance were determined for fixed terms via comparisons to models without the term of interest (see Appendix C for summary output on all global models). Residual plots from all binomial GLMMs were checked using the *DHARMa* package in R (Hartig 2017). Model residuals for the continuous data were assessed for normality, homogeneity of variance, collinearity and influential outliers via Cook's distance.

5.3.5.1 Fish community differences in relation to existing variation in motorboat disturbance

All fish survey data were standardised to abundance per 100 m^2. Convict surgeonfish (*Acanthurus triostegus*) were either absent or appeared as >200 individuals per transect due to their schooling behaviour (Randall 1961); this caused difficulties in statistical analysis due to the resulting violations to test assumptions, therefore they were excluded from the dataset. Species assemblages were compared between high- and low-disturbance sites using an unrestricted one-way nested PERMANOVA (maximum permutations = 9,999), with motorboat disturbance (high, low) as a fixed term and sampling-line (1–20) and repeat (1–3) nested within sampling-line as random factors. The unrestricted PERMANOVA permutates raw data, as opposed to model residuals, and is suitable for one-way ANOVA models and models with small sample sizes. Variation in fish species assemblages between high- and low-disturbance sites was visualised using non-metric Multidimensional Scaling (nMDS) based on a Bray-Curtis similarity matrix of the survey data. Non-metric scaling provides a more straightforward interpretation of the ordination plot, where the closer together two points the greater the similarity; the Bray-Curtis similarity matrix provided a robust measure for use on raw abundance data to calculate the percentage difference between pairs, whilst accounting for the influence of joint absences influencing the similarity score (Clarke and Warwick 2001). A percentage similarity analysis (SIMPER: dissimilarity scores between pairs are averaged to provide a dissimilarity for each species per treatment; Clarke 1993) was carried out to identify particular fish species that consistently contributed to the greatest dissimilarity between high- and low-disturbance sites. The species identified as most influential on the variation in fish assemblages between the high- and low-

disturbance sites in the SIMPER analysis were then assessed in separate GLMMs. Univariate species data were returned to original unstandardised counts in order to fit a Poisson distribution. Total fish abundance, species richness and the Shannon Wiener diversity index (Clarke and Warwick 2001) were calculated for each transect and assessed with GLMMs. All GLMMs incorporated motorboat disturbance (fixed), repeat (random) nested within sampling-line (random) and channel (random) as factors.

5.3.5.2 Experimental manipulation of motorboat disturbance

5.3.5.2.1 Stegastes nigricans behaviour

LMMs were used to analyse continuous data (bite rate & sheltering time) and GLMMs were used for binomial data (sediment clearing & chasing). Models for all response measures included short-term treatment (ambient, motorboat), chronic-manipulation treatment (ambient, motorboat) and their interaction as fixed effects; site was included as a random factor. Models of binomial response metrics also included time in view (to control for bias in likelihood of observing a behaviour with trials where the fish was in frame for a longer period of time) as a fixed factor.

For each response metric, the analysis was split between the pre- and the post-chronic-manipulation periods. An initial analysis of baseline behaviour for the pre-chronic-manipulation period indicated no significant difference in bite rate or sheltering time between fish randomly allocated to the two short-term treatments (LMM: all $\chi^2 = 0.04–1.09$, df = 1, all p = 0.30–0.84) or to the two chronic-manipulation treatments (all $\chi^2 = 0.24–0.69$, df = 1, all p = 0.41–0.62), and no significant effect of their interaction (all $\chi^2 = 0.005–0.95$, df = 1, all p = 0.33–0.94). Similarly, there was no baseline difference in the occurrence of either sediment clearing or chasing behaviour between fish randomly allocated to the two short-term treatments (GLMM: $\chi^2 = 0.14–0.68$, df = 1, p = 0.41–0.71) or to the two chronic-manipulation treatments ($\chi^2 = 0.70–0.73$, df = 1, p = 0.39–0.40), and no significant effects of their interaction ($\chi^2 = 0.33–1.17$, df = 1, p = 0.28–0.57).

5.3.5.2.2 Fish community surveys

Fish survey data were standardised to abundance per 100 m^2, and *A. triostegus* was excluded, as per the boat-channel surveys (above). Variation in species assemblages across pre-manipulation sites was compared in an unrestricted one-way PERMANOVA (maximum permutations = 9,999) with pre-manipulation treatment as the single fixed factor, and visualised with an nMDS, based on a Bray-Curtis similarity matrix. The same analysis was then conducted on the post-manipulation survey data to observe any changes to the community composition between the sites exposed to chronic-ambient and chronic-motorboat treatments. A SIMPER analysis was also carried out on the post-manipulation census data to identify fish species that consistently contributed the greatest dissimilarity in distributions between treatments. Species identified as most responsible for variation between chronic-ambient and chronic-motorboat sites were analysed separately using non-parametric Wilcoxon's signed-rank tests. Total fish abundance, species richness and the Shannon Wiener diversity index were also analysed using non-parametric Wilcoxon's signed-rank tests.

5.4 RESULTS

5.4.1 Fish community differences in relation to existing variation in motorboat disturbance

There was no significant effect of natural motorboat-disturbance level on the total fish abundance (GLMM: $\chi^2 = 0$, df = 1, p = 1), species richness ($\chi^2 = 1.16$, df = 1, p = 0.28) or Shannon Wiener diversity index ($\chi^2 = 0.09$, df = 1, p = 0.77). However, there was a significant difference between the high- and low-disturbance sites in the composition of their fish communities (PERMANOVA: Pseudo-F = 2.69, df = 1, p = 0.03, 9948 permutations). An nMDS plot provided a visual representation of the disparity in the fish community composition between the high- and low-disturbance sites (Fig. 5.3a). A SIMPER analysis identified 24 species cumulatively contributing to 90% of the dissimilarity between high- and low-disturbance fish assemblages (Table C.1, Appendix C; Fig. 5.3b).

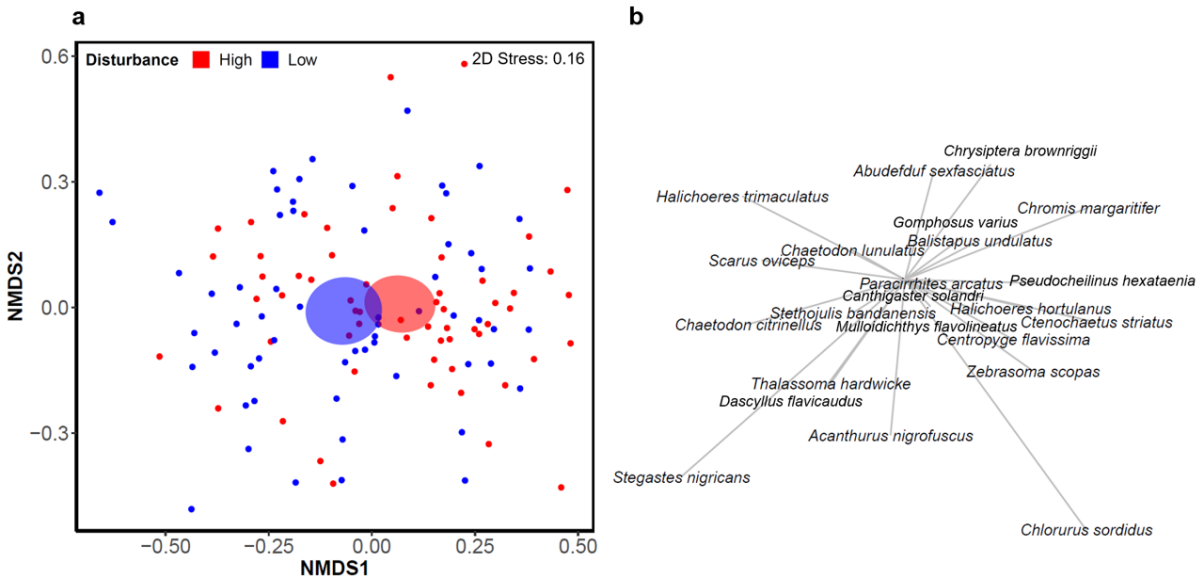

Figure 5.3. (a) Nonmetric multidimensional scaling ordination (nMDS) showing variation in fish community composition between sites exposed to high and low levels of natural motorboat disturbance. Individual dots represent three survey repeats at each site (n = 40 sites); shaded ellipses represent the standard error of the weighted average of each disturbance group. Fish communities from the high- and low-disturbance sites differed significantly (PERMANOVA: Pseudo-F = 2.69, df = 1, p = 0.03, 9948 permutations). (b) Species-loadings plot from the SIMPER output indicating the relative contribution of species to the observed variation in fish community assemblages between sites of low and high levels of natural motorboat disturbance.

Five species were significantly lower in abundance in high-disturbance compared to low-disturbance sites (Table C.1, Appendix C; Fig. 5.4): *Stegastes nigricans* (GLMM: $\chi^2 = 4.81$, df = 1, p = 0.028), *Thalassoma hardwicke* ($\chi^2 = 11.90$, df = 1, $p < 0.001$), *Chaetodon citrinellus* ($\chi^2 = 8.31$, df = 1, p = 0.004), *Gomphosus varius* ($\chi^2 = 14.40$, df = 1, $p < 0.001$) and *Dascyllus flavicaudus* ($\chi^2 = 11.20$, df = 1, $p < 0.001$). Seven species had significantly higher abundances at the high-disturbance compared to low-disturbance sites (Table C.1, Appendix C; Fig. 5.4): *Ctenochaetus striatus* ($\chi^2 = 9.69$, df = 1, p = 0.002), *Chromis margaritifer* ($\chi^2 = 72.50$, df = 1, $p < 0.001$), *Zebrasoma scopas* ($\chi^2 = 15.30$, df = 1, $p < 0.001$), *Centropyge flavissima* ($\chi^2 = 7.70$, df = 1, p = 0.006), *Abudefduf sexfasciatus* ($\chi^2 = 11.70$, df = 1, $p < 0.001$), *Pseudocheilinus hexataenia* ($\chi^2 = 5.94$, df = 1, p = 0.015) and *Mulloidichthys flavolineatus* ($\chi^2 = 12.80$, df = 1, $p < 0.001$).

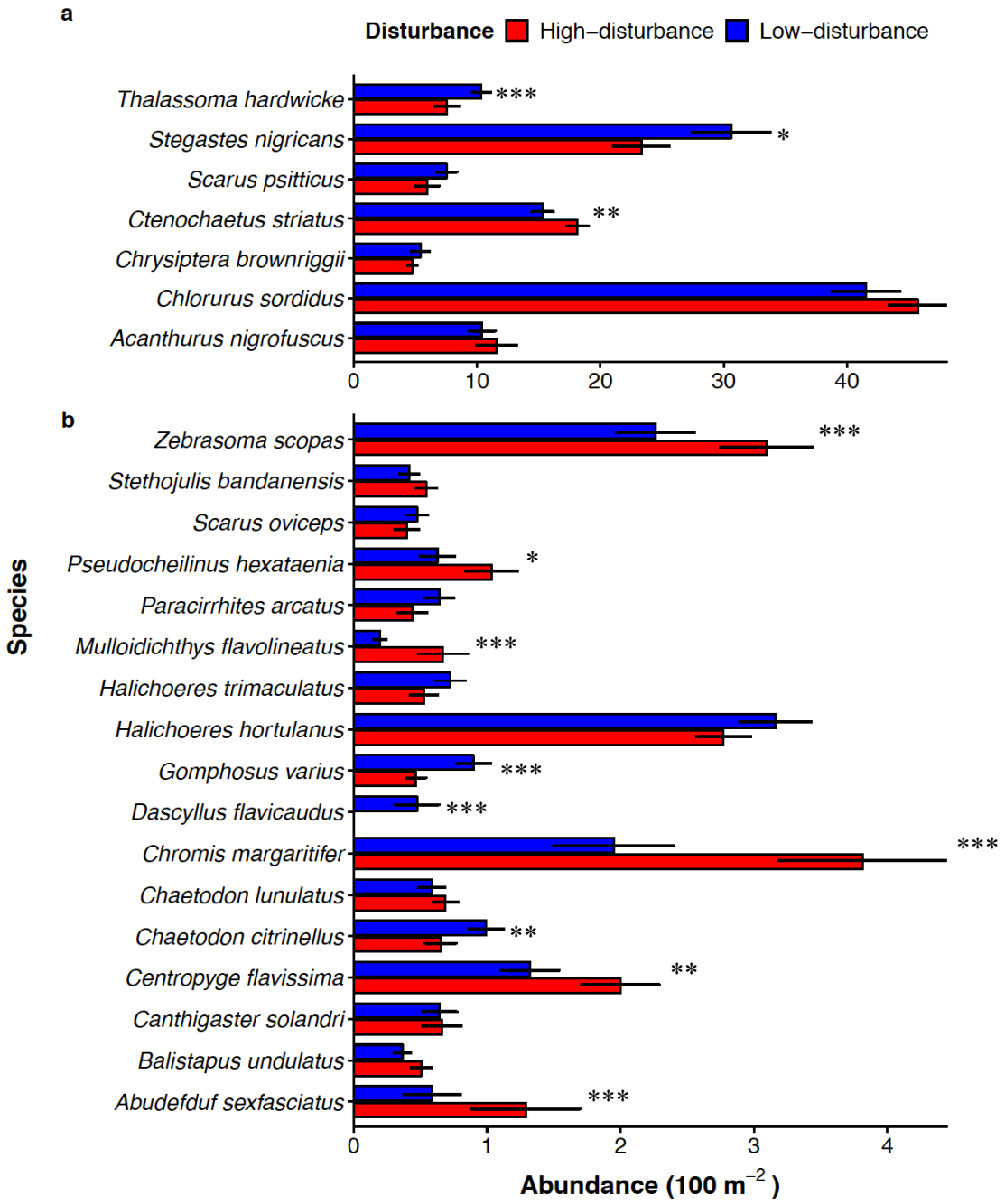

Figure 5.4. Fish abundance per 100 m^2 for each of the 24 species identified from the SIMPER analysis as explaining 90% of the cumulative variation between high- and low-disturbance sites at the boat channels: (a) abundant species (>5 per 100 m^2) and (b) less abundant species (<5 per 100 m^2); * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Bars are means ± SE.

5.4.2 Experimental manipulation of motorboat disturbance

5.4.2.1 Stegastes nigricans *behaviour*

The initial short-term trials before the chronic manipulation revealed no effect of motorboat disturbance. Short-term treatment did not have a significant effect on bite rate (LMM: $\chi^2 = 2.84$, df = 1, p = 0.09; Fig. 5.5a), sheltering behaviour ($\chi^2 = 2.05$, df = 1, p = 0.15; Fig. 5.5b), sediment clearing (GLMM: $\chi^2 = 1.54$, df = 1, p = 0.21; Fig. 5.6a) or the occurrence of chases ($\chi^2 = 0.16$, df = 1, p = 0.69; Fig. 5.6b). As expected, since no chronic manipulation had yet taken place, neither the chronic-manipulation treatment nor its interaction with the short-term treatment had a significant effect on any of the four response variables (full model outputs in Tables C.2–C.5, Appendix C).

Following one month of experimental motorboat disturbance, the influence of chronic exposure on baseline behaviour was first considered. There was no significant effect of chronic manipulation on baseline bite rate (LMM: $\chi^2 = 0.53$, df = 1, p = 0.47), amount of time spent sheltering ($\chi^2 = 0.46$, df = 1, p = 0.50), sediment clearing (GLMM: $\chi^2 = 0.88$, df = 1, p = 0.35) or chasing behaviour ($\chi^2 = 0.39$, df = 1, p = 0.53). As expected, since no short-term treatment had yet occurred at this stage, neither short-term treatment nor its interaction with chronic-manipulation treatment had a significant effect on any of the four response variables (full model outputs in Tables C.2–C.5, Appendix C).

The influence of chronic motorboat exposure on responses to short-term motorboat exposure was then considered. Considering the short-term treatment period following chronic manipulation, there were no significant effects on bite rate (LMM, short-term treatment: $\chi^2 = 0.28$, df = 1, p = 0.60; chronic-manipulation treatment: $\chi^2 = 1.42$, df = 1, p = 0.23; interaction: $\chi^2 = 0.34$, df = 1, p = 0.56; Table C.2, Appendix C; Fig. 5.5c) or the amount of time spent sheltering (short-term treatment: $\chi^2 = 0.77$, df = 1, p = 0.38; chronic-manipulation treatment: $\chi^2 = 0.92$, df = 1, p = 0.34; interaction: $\chi^2 = 1.81$, df = 1, p = 0.18; Table C.3, Appendix C; Fig. 5.5d). Due to a lack of variation in the occurrence of sediment-clearing data, only the chronic-ambient group could be analysed. There was no significant difference in the occurrence of sediment clearing between fish exposed short-term to ambient conditions vs motorboat disturbance (Fisher's exact test: p = 0.66; Fig. 5.6c). There were also no significant effects on the occurrence of chases (GLMM, short-term treatment: $\chi^2 = 0.84$, df = 1, p = 0.36; chronic-manipulation treatment: $\chi^2 = 2.70$, df = 1, p = 0.10; interaction: $\chi^2 = 1.14$, df = 1, p = 0.29; Table C.5, Appendix C; Fig. 5.6d).

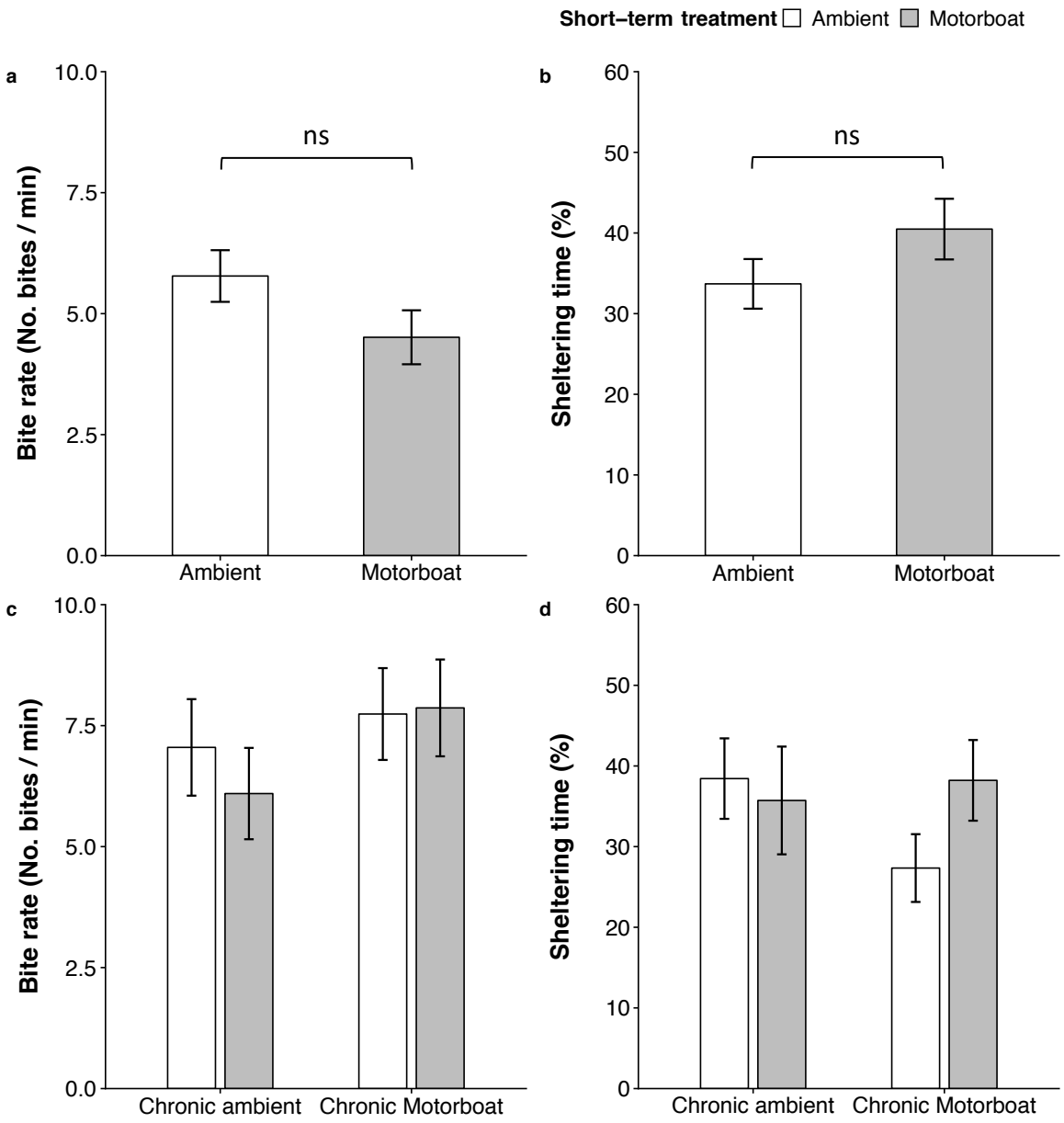

Figure 5.5. *Stegastes nigricans* bite rate and percentage time spent sheltering when exposed to short-term motorboat disturbance or ambient conditions at chronic-motorboat or chronic-ambient sites. Pre-chronic manipulation (a) bite rate ($n_{ambient}$ = 38, $n_{motorboat}$ = 33) and (b) sheltering time ($n_{ambient}$ = 38, $n_{motorboat}$ = 32); values combined for sites assigned to chronic-ambient and chronic-motorboat treatments. Post-chronic manipulation (c) bite rate ($n_{chronic\ ambient/ambient}$ = 18; $n_{chronic\ ambient/motorboat}$ = 16; $n_{chronic\ motorboat/ambient}$ = 20; $n_{chronic\ motorboat/motorboat}$ = 17; LMM, interaction: χ^2 = 0.34, df = 1, p = 0.56) and (d) sheltering time ($n_{chronic\ ambient/ambient}$ = 18; $n_{chronic\ ambient/motorboat}$ = 15; $n_{chronic\ motorboat/ambient}$ = 20; $n_{chronic\ motorboat/motorboat}$ = 17; interaction: χ^2 = 1.81, df = 1, p = 0.18); values split between the four different chronic and short-term treatment combinations. Bars are means ± SE.

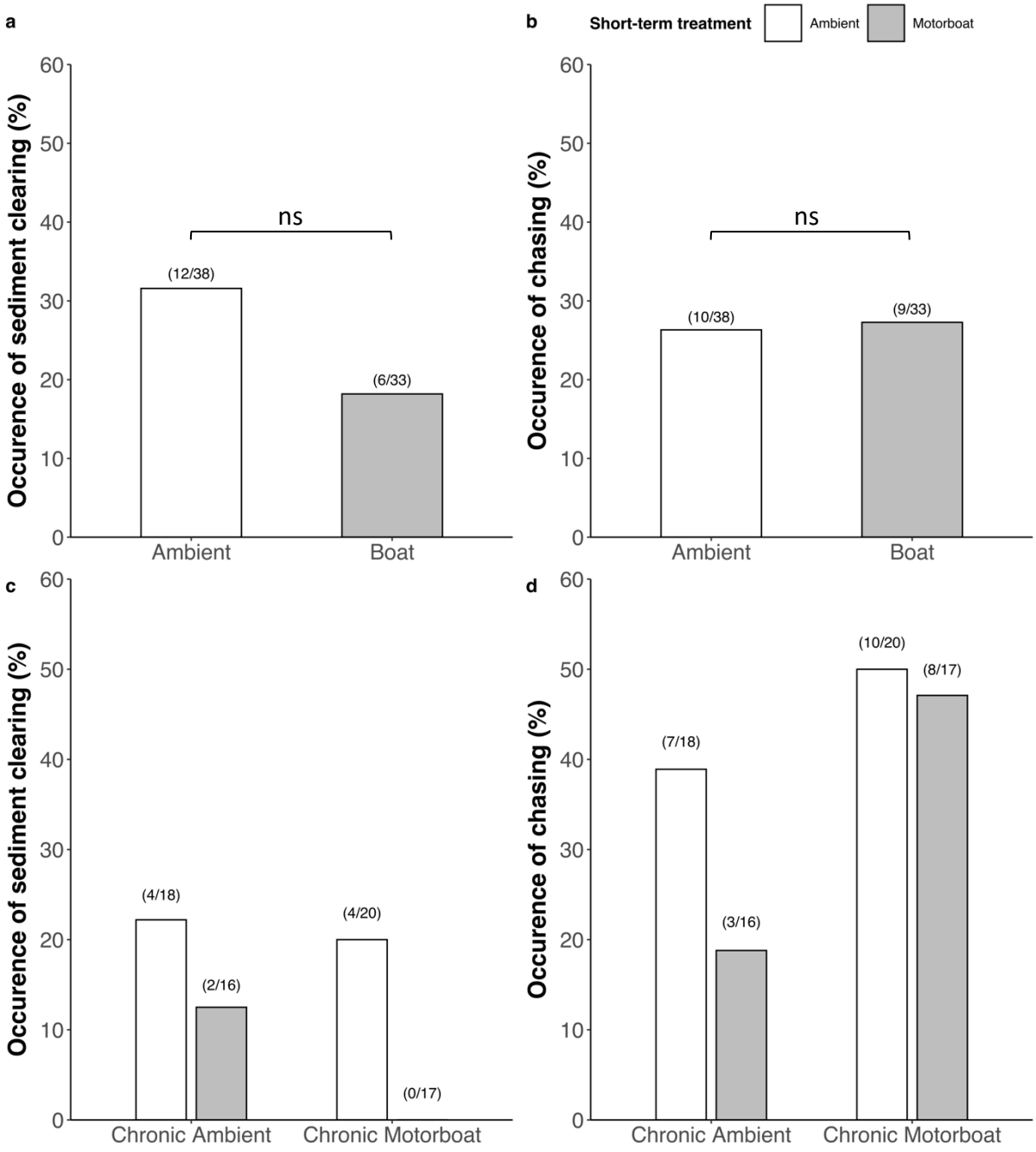

Figure 5.6. Percentage of short-term trials when sediment-clearing and chasing behaviour of *Stegastes nigricans* was observed when exposed to short-term motorboat disturbance or ambient conditions at chronic-motorboat or chronic-ambient sites. Pre-chronic manipulation (a) sediment clearing and (b) chasing. Post-chronic manipulation (c) sediment clearing (Fisher's exact test: $p = 0.66$) and (d) chasing (GLMM, interaction: $\chi^2 = 1.14$, $df = 1$, $p = 0.29$). Values in parenthesis represent the total number of trials where sediment-clearing or chasing behaviour was observed over the total number of trials: $n_{\text{chronic ambient/ambient}} = 18$; $n_{\text{chronic ambient/motorboat}} = 16$; $n_{\text{chronic motorboat/ambient}} = 20$; $n_{\text{chronic motorboat/motorboat}} = 17$ in all cases.

5.4.2.2 Fish community surveys

There was no significant difference between the chronic treatments, before or after the one-month manipulation period, in total fish abundance (Wilcoxon signed-rank test, before: $W = 19$, $n = 6$, $p = 0.94$; after: $W = 18$, $n = 6$, $p = 1$), species richness (before: $W = 16.5$, $n = 6$, $p = 0.87$; after: $W = 18$, $n = 6$, $p = 1$) or diversity (Shannon Wiener index; before: $W = 15$, $n = 6$, $p = 0.70$; after: $W = 13$, $n = 6$, $p = 0.49$). Overall, the fish community composition did not differ significantly between treatment sites before (PERMANOVA: pseudo-$f = 0.457$, $df = 1$, $p = 0.925$) or after (pseudo-$f = 1.41$, $df = 1$, $p = 0.189$; Fig. 5.7a–b) the chronic-manipulation period. An nMDS plot visually indicated the lack of separation in the fish community composition between the chronic-motorboat and chronic-ambient sites following the one-month manipulation period (Fig. 5.7a). In keeping with the boat-channel community census, a SIMPER analysis was used to explore whether some individual species were still affected, despite no significant change in the overall community. SIMPER analysis conducted on post-manipulation survey data indicated that there were 22 species cumulatively contributing to 90% of the dissimilarity that existed in community composition between the chronic-motorboat and chronic-ambient sites (Table C.6, Appendix C; Fig. 5.7b).

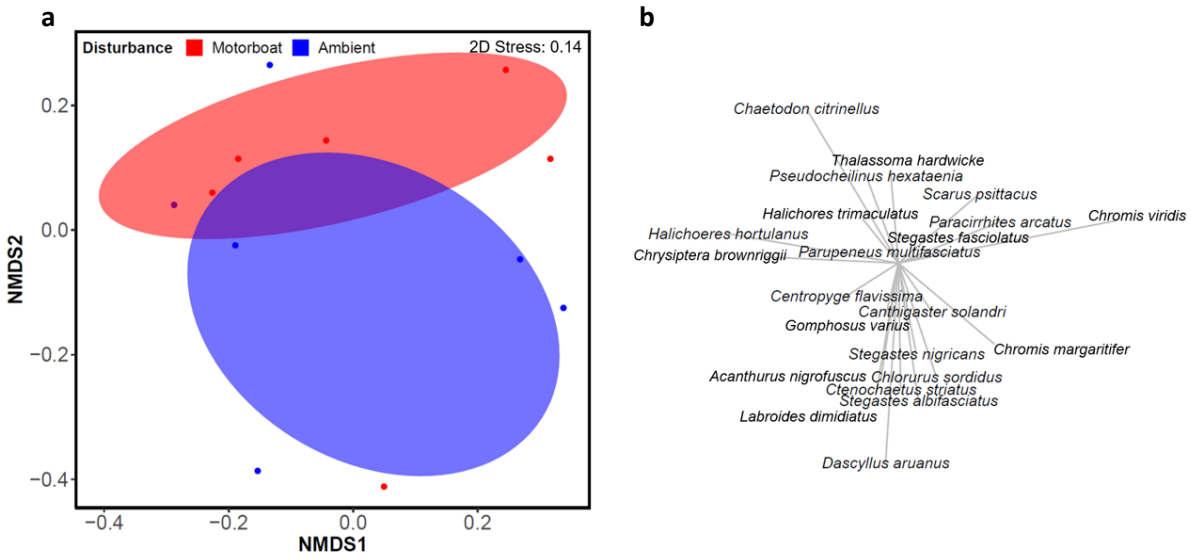

Figure 5.7. (a) Nonmetric multidimensional scaling ordination (nMDS) describing the variation in fish species composition between sites exposed to chronic-motorboat or chronic-ambient conditions. Dots represent transects surveyed at each site (n = 12 sites). Shaded ellipses represent the standard error of the weighted average of each treatment group. Fish community composition did not significantly differ between the treatment sites following the chronic-manipulation period (pseudo-f = 1.41, df = 1, p = 0.189). (b) Species loadings plot from the SIMPER output indicating relative contribution of species to observed variation in fish community assemblages between chronic-ambient and chronic-motorboat exposures.

There were no significant differences in the abundances of any of the 22 species between the treatment sites prior to the one-month manipulation (Wilcoxon signed-rank tests: all $W < 25$, $n = 6$, all $p > 0.05$). However, consistent with the observational findings from the boat channels, there was a post-manipulation decline in *S. nigricans* abundance at the sites exposed to motorboat disturbance compared to ambient sites ($W = 31$, $n = 6$, $p = 0.04$; Fig. 5.8). In addition, there was a post-manipulation increase in *C. margaritifer* abundance at sites exposed to motorboat disturbance compared to ambient sites ($W = 5$, $n = 6$, $p = 0.03$; Fig. 5.8). The abundance of all other species were not significantly affected by the motorboat manipulation (all $W < 28$, $n = 6$, all $p > 0.1$; Fig. 5.8).

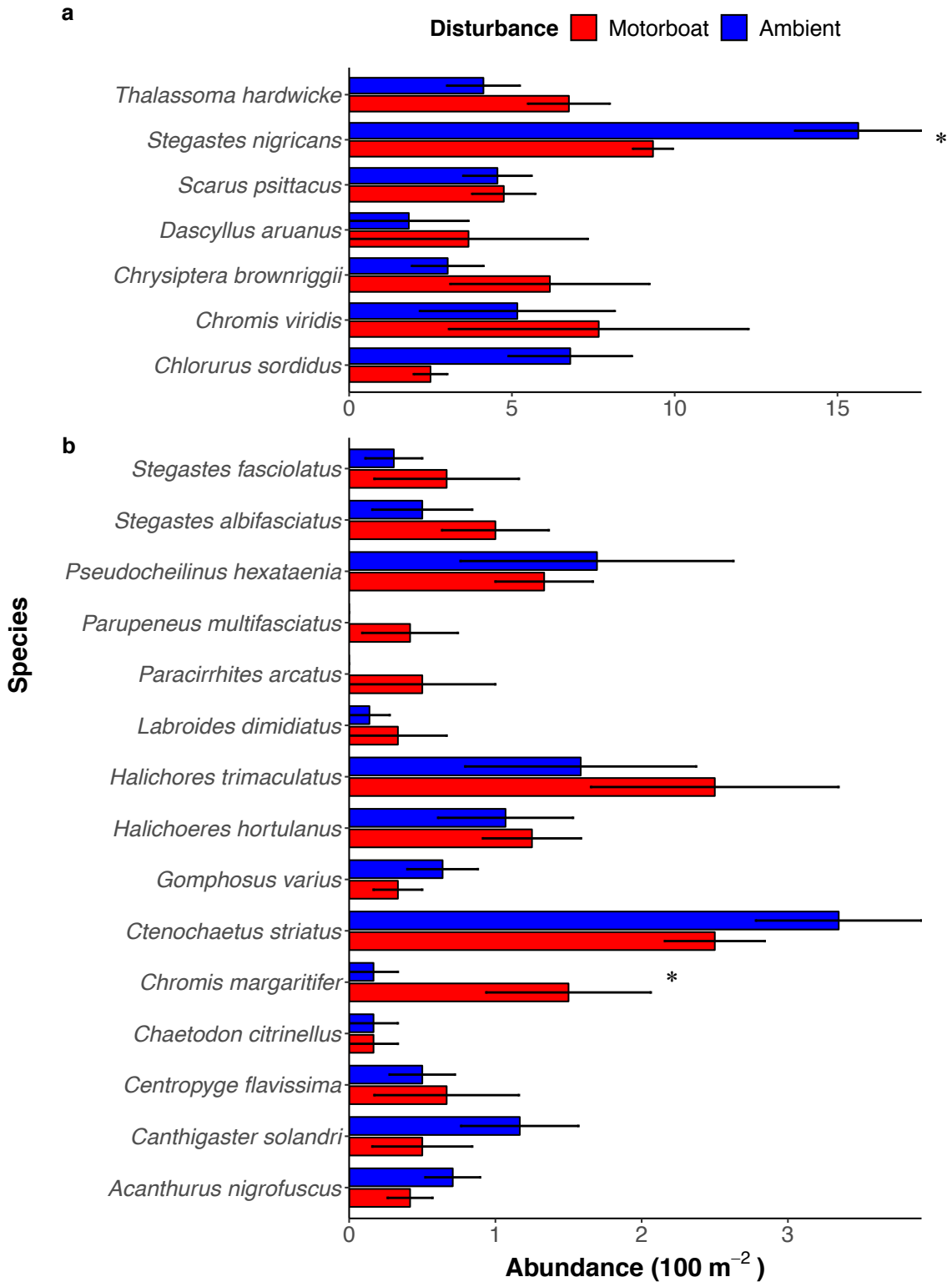

Figure 5.8. Fish abundance per 100 m^2 for each of the 22 species identified from the SIMPER analysis as explaining 90% of the cumulative variation between chronic-ambient and chronic-disturbance sites after the manipulation: (a) abundant species (>5 per 100 m^2) and (b) less abundant species (<5 per 100 m^2); * = $p < 0.05$. Bars are means ± SE.

5.5 DISCUSSION

Twelve common fish species had significantly different abundances in areas of existing high vs low motorboat disturbance, such that the composition of fish was altered significantly depending on the disturbance level. Following chronic manipulation in previously low-disturbance sites by exposure to motorboat disturbance, we replicated the results in two of these fishes, *Stegastes nigricans* and *Chromis margaritifer,* establishing that these changes in abundance could be driven by motorboat disturbance, rather than other potentially confounding factors related to boat channels, such as bathymetric variation and long-term dredging and coral removal. However, changes in the distribution of these two species was not sufficient to significantly alter overall community composition. We found that motorboat disturbance, existing or experimentally manipulated, did not result in any overarching differences in the total abundance, species richness or diversity of the fish community. In addition to the community assessments, we investigated the effects of short-term and chronic motorboat disturbance on one of the species that was found to be affected in the boat-channel community assessments, *S. nigricans*. We found no effects of short-term exposure on a number of behaviours; following the chronic motorboat manipulation, we found no changes in baseline behaviour or in responses to short-term motorboat disturbance. This study, to our knowledge, provides the first experimental evidence of the impact of chronic motorboat disturbance on a fish community.

Anthropogenic noise is a principal component of motorboat disturbance, with previous studies having shown concomitant responses when using real boats and loudspeaker playback (Simpson et al. 2016a; Harding et al. 2018). Species vary in their ecology, life histories (Thorson et al. 2017), hearing sensitivities (Popper and Fay 2011) and scope for plasticity in responses (Sih 2013; Wong and Candolin 2015), which all likely influence how they respond to anthropogenic noise. Changes in fish community composition identified in this study were evident only at the species level rather than at broader ecological metrics (total abundance, species richness and diversity), suggesting that impacts of noise may be complex and difficult to predict. This contrasts with terrestrial studies, where overall bird species richness can be lower in noise-disturbed habitats (Francis et al. 2009) and higher reproductive success is made possible for some species in noisy habitats due to disrupted predator–prey interactions (Francis et al. 2009; Herrera-Montes and Aide 2011). It is important to note that the reefs within the north fringing reef-lagoon system of Mo'orea lack diversity compared with healthier nearby reefs, due to a history of spearfishing, degradation from agricultural runoff, crown-of-thorns outbreaks, cyclones and coral bleaching (Faurea 1989;

Gillett and Wayne 2006; Chin et al. 2011; Rouzé et al. 2015), and so the common species found here more heavily dominate the reef community, and may have higher tolerance to stress. Noise, therefore, may have wider effects on healthier reef communities that are less altered by human influence and disturbance history.

Stegastes nigricans had a 24% lower abundance at existing high-disturbance compared to site-matched low-disturbance sites. This is the most common species in this reef-lagoon system and so its disparity in abundance was responsible for driving the greatest observed difference between the high- and low-disturbance communities. *Stegastes nigricans* is a highly soniferous species producing low-frequency pops and pulse trains, with acoustic communication playing a central role in reproduction, feeding, nest defence and predator avoidance (pers. obs. H.R.H. & E.W.; described in other *Stegastes* spp. by Weimann et al. 2018). Thus, factors that reduce vocalisation efficiency and function may have indirect deleterious fitness consequences. Auditory masking—whereby sound level and frequency of an artificial noise directly competes with the natural biotic sounds emitted by marine fauna—represents one such mechanism (Fay and Megela-Simmons 1999; Brumm and Slabbekoorn 2005). It is possible that the high noise levels associated with the boat channels could mask courtship calls, sounds of intruders, conspecific alarm calls signalling predatory threats, and aggressive and deterrent vocalisations (Radford et al. 2014). Motorboat noise has been shown to affect predator–prey dynamics in another coral reef damselfish, *Pomacentrus amboinensis*, resulting in increased mortality by predation (Simpson et al. 2016a); increases in predation could underpin the change in abundance of *S. nigricans* found in this study. Additionally, changes in *S. nigricans* population distributions may also be driven by direct impacts on settlement-stage larvae, as previously found in cardinalfish and damselfish larvae (Holles et al. 2013; Simpson et al. 2016b), whereby exposure to motorboat noise reduced the natural ability of larvae to move towards reef sound – a key settlement cue for larval coral reef fish (Simpson et al. 2005; Holles et al. 2013). Recruitment success would not explain the change in abundance following the chronic manipulation, but it could, over multiple generations, contribute to the long-term alterations to *S. nigricans* distributions.

Two common predators on this reef system, the sixbar wrasse (*Thalassoma hardwicke*) and the Pacific bird wrasse (*Gomphosus varius*), were also observed at significantly lower abundances in high-disturbance areas. While there has been substantial focus on the detrimental impact of anthropogenic noise on vigilance and predator avoidance in prey species (Bruintjes and Radford 2013; Simpson et al. 2015; Purser et al. 2016; Spiga et al. 2017; Ferrari et al. 2018; McCormick et al.

2018b), few studies have addressed impacts on predators (for exceptions, see: Simpson et al. 2016a; McCormick et al. 2018a). These studies have generally assessed the capture success of a predator when hunting in noisy conditions. However, the effects of motorboat noise on the ability of a predator to seek out and correctly identify prey in the wild, which is arguably more cognitively demanding, are unknown. Predators may rely heavily on sound generated by prey during hunting (Holt and Johnston 2011), which could make them more susceptible to detrimental noise effects than herbivores, detritivores and planktivores, causing them to move to quieter environments.

Community-level responses to an environmental disturbance are usually the result of interference with functional traits and complex interspecific interactions, rather than direct equal impacts on all species of the community (Skagen et al. 2006; Williams et al. 2010). Adult *T. hardwicke* prey upon the eggs of *S. nigricans* (Shima and Osenberg 2003). Thus, their distribution may be driven by *S. nigricans* abundance, which may explain the lower abundances of *T. hardwicke* in the high-disturbance areas. If this is indeed the case, once *S. nigricans* populations decline in response to the introduction of noise we could expect a lag before *T. hardwicke* distributions follow, as expected given classic theory on predator–prey dynamics (Lotka 1925). The lack of response from *T. hardwicke* to motorboat disturbance observed in the month-long motorboat manipulation would support this hypothesis; but, if the manipulation was continued for longer, *T. hardwicke* distributions may follow a similar trend to *S. nigricans*. This indirect response would be the first indication that motorboat disturbance could trigger trophic cascades within a marine community.

Some species were found to have a greater abundance in areas of high motorboat-disturbance compared to areas with to low acoustic disturbance. *C. margaritifer, C. flavissima, A. sexfasciatus* and *P. hexataenia* all had significantly greater abundances in areas exposed to motorboat disturbance. Like *S. nigricans,* all these fishes are site-attached, meaning that they remain tightly associated to small areas of reef. However, unlike *S. nigricans*, these species do not farm turfing algae, and so have less requirement to defend their territory aggressively. Thus, in areas of high *S. nigricans* densities, these less aggressive species are likely outcompeted for space. As a consequence, where high motorboat disturbance leads to lower *S. nigricans* abundance, the alleviated competition for space, along with potentially reduced predation pressure, could allow these sedentary, but potentially more noise tolerant, species to persist in greater abundance. Similar interactions have been shown in a terrestrial system; birds nesting in noisy sites were shown to have a higher reproductive success than those from quiet areas, as a result of reduced nest predation due to the predators intolerance to noisy locations (Francis et al. 2009).

Two common acanthurids, the striated surgeonfish (*Ctenochaetus striatus*) and the brushtail tang (*Zebrasoma scopas*), were also observed in significantly greater abundances in areas of high-disturbance. Acanthurids produce sounds during courtship and agonistic interactions (Tricas and Boyle 2014), but do not rely on sound in order to forage on algae and detritus. Moreover, they appear to possess relatively low hearing sensitivity (Colleye et al. 2016), which may confer greater tolerance to noise than other families. *Ctenochaetus striatus* and *Z. scopas* likely experience agonistic chases from *S. nigricans* as their diets contain elements of the epilithic algal matrix (EAM)—detritus, sediment, filamentous algae and invertebrates (Wilson and Bellwood 1997)—that is maintained by *S. nigricans* inside their territories. Thus, reefs with fewer *S. nigricans* may present *C. striatus* and *Z. scopas* with more opportunities to graze on unguarded EAM, alleviating competition for food.

Despite the finding that *S. nigricans* distributions were affected at both existing and experimentally manipulated motorboat-disturbance sites, there was no observable change in individual *S. nigricans* behaviour following chronic motorboat disturbance. This contrasts with previous studies on pomacentrids that have found behavioural effects during short-term exposure to motorboats (Simpson et al. 2016a; Holmes et al. 2017). It is not likely that the motorboat noise stimulus was inaudible, as *S. nigricans* is highly soniferous and is likely to have relatively good hearing (see Kenyon 1996 for a congeneric audiogram). However, the behavioural response metrics chosen may not have been sensitive to noise; other metrics, such as anti-predator responses (e.g. Simpson et al. 2016a), may have shown changes as has been found in other species (Simpson et al. 2015; Spiga et al. 2017). The lack of an effect of motorboat noise on short-term behaviour, but strong evidence for altered distributions, highlights the complex nature of noise effects, suggesting that measuring species-specific responses in isolation could be misleading.

Species vary, with their ecology, life histories, hearing sensitivities and capacity for phenotypic change likely dictating winners and losers in environments polluted by anthropogenic noise. Future work could explore whether species remaining in noisy habitats suffer any trade-offs; e.g. reduction in growth rates or fecundity (Slabbekoorn et al. 2010). Here, we have shown that chronic motorboat disturbance can affect coral reef fish distributions. To date, the majority of studies have only assessed short-term effects of anthropogenic noise on single species or simple interspecific interactions. This study highlights the need for caution when translating impacts of anthropogenic noise on single species to community-wide consequences, with interspecific variation

in noise tolerance combined with multiple interspecific interactions likely to govern community-level change in response to chronic noise disturbance. Understanding responses to chronic and repeated noise exposure of individual species, their potential for habituation and their interactions with other members of the community, is key to predicting future ecosystem functioning and stability in the face of this pervasive pollutant. This will develop a stronger basis to manage global acoustic disturbance of vulnerable ecosystems, by implementing mitigation policies in boating zones and engineering low-disturbance engine and propeller systems, with the ultimate goal of reducing our acoustic footprint on the marine environment.

Chapter 6 – General discussion

Contributions to the work

H.R.H conceived the ideas, wrote the chapter, and revised the chapter following comments by A.N.R.

6.1 OVERVIEW

Underwater anthropogenic noise is a pollutant of global concern, with wide-ranging effects on multiple taxa across many biological levels of organisation (Slabbekoorn et al. 2010; Kunc et al. 2016). Over the last 15 years, research has shown noise to cause physical injuries (e.g. ruptured swim bladders, haematomas) close to, particularly intense, impulsive, sources; further from the source, physiological and developmental effects (e.g. stress responses, developmental abnormalities, reduced growth rates) are evident. At much lower sound levels, and thus the greatest distances from the source and covering the greatest area, behavioural effects (e.g. alterations in parental care, foraging, anti-predator responses) are apparent. However, the majority of organismal-impact studies to-date have considered responses using combined population means, not accounting for the variation that exists between individuals (Chapter 1; Radford et al. 2016a). Individuals within a population can vary depending on intrinsic characteristics (e.g. size, sex, personality, body condition) and extrinsic factors (e.g. environmental context, prior experience). Intraspecific variation underpins evolution, and can directly influence population dynamics, community structure and ecosystem functioning. Failing to consider this variation risks under- or over-estimating the full impacts of noise exposure, and jeopardises mitigation strategies to reduce the impact of this pervasive pollutant. Here, I summarise the main findings from each chapter and suggest additional areas of research directly stemming from them. More broadly, I provide suggestions for future work that would enhance our understanding of the consequences of anthropogenic noise for wildlife. Finally, I discuss existing and emerging strategies and technologies that may help 'us', as a global society, to minimise the impact of noise pollution on our aquatic habitats.

6.2 CHAPTER SUMMARIES

I started by carrying out a systematic literature review to investigate what is already known about intraspecific variation in non-human animal responses to anthropogenic noise. I found that less than 10% of studies experimentally testing responses to noise of both terrestrial and aquatic organisms considered the variation that exists between individuals within a population or species. The majority of studies experimentally considering intraspecific variation used fish as model organisms (42%), followed by birds (27%), mammals (15%), arthropods (10%), and amphibians (6%). The influence of extrinsic factors on responses to noise has been researched the most (71%), with repeated exposure and environmental context being most commonly studied. Less work has considered intrinsic

characteristics; those studies that have done so focussed on sex, body size, body condition and personality. Of all experimental studies considering intraspecific variation in responses to noise, 75% report significant effects, with equivalent levels of significance between intrinsic characteristics and extrinsic factors. With fewer than 10% of studies considering intraspecific variation it is likely that we could be over- or under-estimating the full impacts of anthropogenic noise.

My first data chapter (Chapter 2) focussed on developing methodologies which would later be used for *in situ* testing of fish responses to real sources of anthropogenic noise. I investigated the acute effects of playback of pile-driving noise on Atlantic salmon (*Salmo salar*) behaviour and physiology. I found no short-term effects of the additional noise, but provide a number of caveats to the research that indicate the importance of testing wild fish, ideally in open-water or natural conditions, to gain an accurate understanding of the impacts. In this chapter, I was working alongside Marine Scotland Science (CASE partner), from which I benefited greatly through learning about the needs of end-users. For instance, I reported my findings to a board of stakeholders (including members from salmon fisheries boards and industry).

In Chapter 3, I investigated how body condition and habitat quality influence responses of the blue-green damselfish (*Chromis viridis*) to motorboat noise. I found that fish in poorer condition performed worse than those in better condition, in terms of their anti-predator response to a looming stimulus, during noise exposure. This difference is likely to have fitness consequences, as poorer condition fish only startled when the stimulus was closer to them, which potentially translates into a higher risk of predation. I found no significant effects of motorboat noise on a physiological metric, opercular beat rate, when comparing fish of different relative condition and those from healthy and degraded habitats.

Chapters 4 and 5 focussed on whether prior experience can influence responses to real motorboat noise. In Chapter 4, I found that endemic cichlid fish (*Cynotilapia zebroides*) living in historically high-disturbance habitats have a reduced sensitivity to motorboat noise compared to fish from low-disturbance sites. Such a difference might occur within a generation – through altered tolerance, phenotypic plasticity, changes in hearing thresholds or evasive emigration of more sensitive individuals – or over multiple generations through selection of more tolerant genotypes. To address some of these possibilities, I investigated whether individual dusky farmerfish *(Stegastes nigricans*) exhibited a changed response to motorboat noise following one month of experimental disturbance in natural conditions (Chapter 5). I found that individual *Stegastes nigricans* behavioural

responses were not affected by short-term noise exposure from motorboats, and there was no change in baseline behaviour or responses to noise following chronic experimental manipulation of motorboat disturbance.

Chapter 5 also expanded from consideration of just single-species responses or simple interspecific interactions, to determining the effects of chronic motorboat noise on an entire community; this approach is vital if we are to understand fully the effects of anthropogenic noise on entire ecosystems. I first carried out an observational study using existing variation in motorboat traffic, and then assessed community changes following experimental manipulation of chronic motorboat noise. Existing spatial variation in motorboat disturbance was related to a difference in community structure, with some species more or less prevalent in high- and low-disturbance sites. These interspecific differences were likely driven by differences in auditory sensitivity, tolerance and complex interspecific interactions. Crucially, I was able to replicate some of the species differences seen between the two disturbance levels in a chronic (one month) experimental manipulation. Following long-term motorboat disturbance, there were changes in the abundances of two of the species found to differ in the observational study at sites of different natural disturbance levels.

6.3 FUTURE RESEARCH

In Chapter 3, I found no difference in the opercular beat rate of fish from degraded and healthy coral habitats when exposed to motorboat noise, but testing required confinement of the fish in small containers. Previous research has shown a significant difference in anti-predator performance of groups of *Pomacentrus moluccensis* living on degraded and healthy host coral colonies (Boström-Einarsson et al. 2018). As such, it would be interesting to test responses of fishes from varying habitat qualities to noise *in situ*, using free-swimming behavioural metrics. In addition, the ideal would be to assess fitness consequences directly rather than from proxies such as the responses to a simulated predatory strike (a looming stimulus in my experiment). This may involve investigating real predator–prey interactions *in situ* of a host coral colony that varied in habitat quality, which either differed naturally or was experimentally manipulated. In addition, I was only able to infer chronic stress from high ventilation rates, and was not able to measure baseline cortisol. Establishing baseline cortisol levels is important as chronic stress can influence responses to further stressors, e.g. potentially through down-regulation of the primary stress axis due to the elevated circulating cortisol (Barton et al. 2005). Future work may look at raising the baseline cortisol level using implants (e.g. Lawrence et al. 2019), and then subsequently test fish to motorboat disturbance

to determine whether the magnitude of response was related to pre-exposure baseline cortisol levels.

In chapter 5, I aimed to test individual responses to motorboat noise, and then determine whether responses change following a period of chronic motorboat disturbance. I found no effects of short-term noise which meant I was not able to address whether responses can change within a generation. The development of new genetic-sequencing technologies, such as Restriction site-Associated DNA sequencing, will enable the rapid assessment of underlying genotypic variation among populations (for an example of where this technology as been used with respect to other anthropoegnic disturbances, see: Paris et al. 2015); this would help identify changes within populations exposed to chronic noise even if there are no discernible alterations in behaviour. Further, it is still currently unknown whether parental experience of noise is transferred to the offspring, as has been observed in response to high CO_2 conditions (Allan et al. 2014). Elevated CO_2 reduced reactivity and locomotor activity in juvenile coral reef fish; however, in juveniles where the parents were exposed to high CO_2, effects were reduced in some behavioural traits (Allan et al. 2014). Understanding such trans-generational effects would help to determine whether and how populations can adapt to chronic noise.

Determining how organisms may respond to chronic noise exposure is needed to develop our understanding of whether individuals can adjust their physiology and behaviour to maintain fitness. Chapter 4 was able to show the importance of acoustic history in responses to motorboat noise; however, it was not possible to determine over what period of time such a change might occur. It would also be interesting to see whether all species can show some level of selection or plasticity (altered tolerance) to chronic noise, as was indicated in the cichlid *Cynotilapia zebroides* in Chapter 4. Previous research has already shown interspecific variation in responses to noise; three-spined sticklebacks (*Gasterosteus aculeatus*) made more mistakes when foraging for *Daphnia magna*, whereas European minnows (*Phoxinus phoxinus*) reduced their foraging effort (Voellmy et al. 2014a). In addition, sites located next to gas wells with noise-producing compressors were shown to alter community composition compared to control sties, with changes due to species-specific responses to noise (Francis et al. 2009). In Chapter 5, I showed that not all fish species respond in the same way to chronic noise exposure, with some more abundant at high-disturbance sites (cf. low-disturbance sties) and vice versa. As such, interspecific variation in the capacity for adaptation and behavioural plasticity, and what it is about particular species that may make them more or less vulnerable to noise, is key for understanding future communities. Furthermore, determining if those

species that increased in abundance under noisy conditions are 'winning' the ecological contest when exposed to anthropogenic noise is important to consider. It is possible that the, potentially, more tolerant species that remain in noisy sites are making trade-offs in growth or altered fecundity (Slabbekoorn et al. 2010).

A more thorough understanding of the auditory thresholds of different fishes *in situ*, in both the pressure and particle-motion domains, is needed (Popper and Hawkins 2019). This would enable better impact assessments and improved model predictions on the potential effects of noise on fishes. To date, a lot of assumptions are made on the likely hearing ranges of different species based off their range of vocalisations. Further, more work on the potential ontogenetic shifts in hearing thresholds (Kenyon 1996) would enable greater consideration of intraspecific differences in responses to anthropogenic noise. Along with more robust measurements of fish hearing, the adoption of a standardised way of reporting acoustic metrics across biological studies is needed to aid in interpretation and facilitate comparisons. A standardised methodology in reporting acoustic metrics across studies would make meta-analyses more feasible (Shannon et al. 2015), and aid in determining likely threshold levels for a range of biological responses (e.g. physical injuries, physiology, and behavioural impacts), for use in regulation of anthropogenic noise. Initially, limits may be based on the worst-case scenario for those species that are the most sensitive, which can subsequently be refined as new information comes to light (e.g. adjusted depending on the environmental context, the body condition of the inhabitants, or the acoustic history of the area).

6.4 MANAGEMENT OF MOTORBOAT NOISE IN AN INCREASINGLY NOISY WORLD

The more we develop our understanding of the impacts of underwater anthropogenic noise, the more this knowledge can feed into safeguarding our aquatic environments. Noise pollution from commercial shipping, seismic surveys and offshore construction (e.g. pile-driving) has received the most widespread attention (see Merchant 2019 for a recent discussion on policy and noise abatement for these activities) and, as such, have the most comprehensive 'guidelines' focussed on managing and reducing the acoustic footprint of these sources. Yet, in many coastal regions, small motorised vessels (e.g. recreational motorboats, inshore fishing fleet, personal water craft) can make up the majority of all boating activity in many areas (Whitfield and Becker 2014). On the Great Barrier Reef, the use of small motorboats is forecast to increase dramatically by 2050 (Great Barrier Reef Marine Park Authority 2014), and pleasure craft are the fastest growing marine sector in the arctic region (Johnston et al. 2017). Legislation has not maintained pace with this rapidly growing

source of anthropogenic noise that can have a wide reach in coastal environments; motorboats are able to go where larger vessels cannot, including marine protected areas (MPAs) (Bittencourt et al. 2014; Dinh et al. 2018). Despite being areas most protected from degradation, some MPAs have seen large increases over the last decade in the number of boat visits (Gonson et al. 2016). Whilst many of the outlined 2014 guidelines by the International Maritime Organisation (IMO) to regulate commercial shipping may apply to small vessels, the latter also present unique challenges and management approaches (Johnston et al. 2017). Here, I discuss potential options that could be used to manage motorboat use at a local scale.

6.4.1 Local area management

The use of speed restrictions in designated areas may be one way in which to reduce the noise radiated from small motorboats. There has been much attention on the use of 'slowdowns' for commercial shipping. For instance, cumulative noise from transiting ships was much lower when there was a 65% reduction in operational speed to eight knots (McKenna et al. 2013). Acoustic models suggest that by setting ship speed restrictions to 11.8 knots (affecting 83% of the shipping fleet that travel faster than that speed restriction) would reduce emitted noise in the Haro Strait (Salish Sea) by 3 dB (Williams et al. 2019). These options are, however, very dependent on ship type and design: in general, emitted noise increases with speed and size of vessel, yet some types of ships get louder as speed is reduced (McKenna et al. 2013; Veirs et al. 2016). The same principle could be applied for smaller vessels but, to my knowledge, there has been limited assessment on the characterisation of sound from multiple boat types and speeds (for exceptions, see: Erbe 2013; Brooker and Humphrey 2016). Thus, there is a need to establish baseline sound levels for different types of boat. This would require extensive acoustic monitoring for a range of boat types, but is something that could be built into the design stage and in order to market the craft the company need to include an acoustic analysis for different operational speeds. However, when imposing speed restrictions there is a trade-off between intensity and duration of sound exposure if slowing down; it may be that as a result of slowing down, cumulative noise levels may not be much different to moving through the area faster, which would result in less efficient transport and higher fuel costs. Speed restrictions in national parks have proven effective in some scenarios and can reduce overall degradation of an acoustic habitat (McKenna et al. 2017), but much more research is needed in this regard.

In addition to the use of speed restrictions to reduce the noise radiated from small motorboats, spatiotemporal restrictions may represent an important asset for local area managers to control the activity of small motorboats in certain areas. This encompasses a range of options, including closure of particular areas during sensitive times of year (e.g. known spawning seasons), as well as providing a buffer zone around the site. The creation of a buffer zone should be based on empirical research. However, there is currently a paucity of data considering the spatial scale of noise impacts (does-response relationships) on fishes. Greater consideration of the spatial scale of impact from motorboats would go a long way in allowing managers and policy makers to set meaningful spatial restrictions to help protect marine life. Further, to reduce overall activity within an area, and subsequent acoustic degradation, managers could use a permit system. Permits could be required for small recreational motorboats such that they only be allowed into sensitive areas if they conform to certain guidelines, or the vessel meets certain criteria as part of a certification scheme. One example of such area-based management is that of the US National Park Service with respect to vessel activities inside Glacial Bay (Alaska). Here, they use a permit system to regulate the number of entries into the bay, as well as impose speed limits (www.nps.gov).

6.4.2 Public perceptions and voluntary guidelines

Public attitudes are often a key step in driving change in governance and policy. Increasing public awareness of underwater noise pollution would be beneficial for motorboat users; informing them about ways to limit their impact in the absence of legally binding national or international regulations. Voluntary guidelines may include reducing speed if close to reefs or MPAs, or passing further away. Engine choice for those purchasing a new boat or in need of a new engine may be an important consideration. For instance, engine-type has been show to affect the responses of coral reef fishes to noise exposure, with a reduced response to 2-stroke vs 4-stroke combustion engines (Jain-Schlaepfer et al. 2018; McCormick et al. 2018b, 2019). This kind of research, if made widely available, gives managers a useful tool in regulating areas and provides environmentally conscious members of the public important knowledge when purchasing a new boat or engine. In addition, there are suggestions that cleaning and maintaining of boats helps to reduce the radiated noise. Alternatively, managers and regulators may impose restrictions on certain engine types. For example, to phase out particular types or to designate particular areas only for particular engines that have been shown to have a reduced impact through experimental testing. Electric engines may serve to reduce radiated noise from the operation of the engine, although reduction in noise levels stemming from cavitation would still be needed.

6.4.3 Baseline acoustic monitoring

Understanding baseline levels of ambient noise, both spatially and temporally, is urgently required in order to best legislate and protect marine habitats. The Ocean Noise Reference Station Network is one such monitoring effort aimed at developing this knowledge (Haver et al. 2018). Yet, it would be beneficial if this was applied at local scales to monitor small motorboat use, which varies spatially and temporally. Understanding the pervasiveness of motorboat use is needed, and is something rarely reported for many areas (Gonson et al. 2017); there is a real need for multiple *in situ* noise measurements (Dinh et al. 2018), which could greatly help local area managers and regulators in designing and proposing management strategies. Motorboat activity has been shown to be positively correlated with coral cover and fish density, indicating that often the most high-quality and biodiverse habitats which require protection are, potentially, under the most stress; it is therefore important to monitor boating activities in sensitive areas (Venturini et al. 2018). This would help to identify particular habitats at greater risk of acoustic degradation, and give support to understanding the cumulative impacts of noise in certain areas. Further, it would be beneficial if such long-term monitoring was recorded in both particle motion and pressure. Knowledge of the former is critical for fish behaviour, yet the majority, if not all, of Passive Acoustic Monitoring (PAM) is done in the pressure domain only.

Characterising the spatiotemporal use of motorboats around coastlines would be a large step forward in determining areas that may require better protection. The development of baseline monitoring at local scales or improved acoustic modelling from small motorboats, combined with spatial distribution maps for sensitive species or species-specific habitat quality, would benefit spatial planners in identifying areas to prioritise for mitigation or small-scale regulation (Erbe et al. 2014). Further, this information could enable the use of risk-based management strategies that have been previously suggested for commercial activities (Merchant et al. 2018). This methodology could be adapted for use at local scales for the control of motorboat noise to understand the cumulative and aggregate exposure of certain areas. This may indicate sensitive time periods or particularly important coastal habitats to prioritise protection and impose restrictions on transits, or when managers can apply spatial exclusions or buffer zones. Additionally, information about the cumulative noise exposure of particular areas could be used to infer the acoustic history of resident populations and thus determine whether responses to noise may be altered as a result (Harding et al. 2018). This information could also be used during environmental impact assessments (EIASs) as

an additional consideration when determining the risk of adverse impacts from anthropogenic noise on individuals and populations.

6.4.4 Emerging technologies

Motorboat use might be particularly difficult to regulate if it is not focussed on particular areas, such as marine parks or MPAs. However, solutions to reduce the noise emitted from motorboats altogether will help lessen the problem. Innovative technologies represent an encouraging avenue for development in reducing the acoustic energy released into marine environment. A significant portion of the noise produced from motorised vessels stems from propeller cavitation; as such, considerable efforts are focussing on ways to reduce this (e.g. coatings, propeller design) (Ebrahimi et al. 2019). Oscar Propulsion, a UK-based company, have recently developed a PressurePores system which is claimed to reduce the radiated noise from the propeller by 17–21 dB (http://oscarpropulsion.co.uk/). Continued development of technology like this provides great optimism for reducing our overall acoustic footprint. Other research has focused on increasing the speed at which cavitation occurs (cavitation-inception speed), through methods such as injecting water around the propeller tips through purpose made ducts (Lee et al. 2018). Although, tests show only significant reductions between 2000 and 4000 Hz, so development is needed if such technology is to reduce the sound levels within the hearing ranges of many fishes. The use of electric engines is an enticing prospect, especially for small vessels, and is likely to result in reduced machine noise. Yet, without development of propeller design to reduce radiated noise it is possible reductions will be minimal. Some electric motorboat designs have been shown to be quieter than a boat with a widely used combustion engine (Nishimura et al. 2013). Establishing the effects from currently available electric outboard engines on marine life would be worthwhile testing.

6.5 CONCLUSION

In this thesis, I have provided experimental evidence of intraspecific variation in responses of fishes to motorboat noise. With the global human population continuing to rise, and the number of people living within touching distance of the sea increasing, recreational motorboat use, as well as other noise-generating human activities, are predicted to increase considerably. As such, it is imperative that we understand fully the impacts that anthropogenic noise can have on marine life. All individuals are unique, shaped by the habitat they call home and their individual characteristics. Continued incorporation of intraspecific variation into the study of anthropogenic noise will greatly

improve the mitigation and management strategies used to safeguard marine fauna, produce more realistic models of species responses to noise, and further enhance the monitoring processes used to assess effects on populations. In the current climate crisis we find ourselves in, it is encouraging that we can reduce our acoustic footprint almost overnight and help alleviate some of the pressures on an already pressurised world.

References

Afonso LOB, Basu N, Nakano K, Devlin RH, Iwama GK. 2003. Sex-related differences in the organismal and cellular stress response in juvenile salmon exposed to treated bleached kraft mill effluent. Fish Physiol Biochem. 29:173–179.

Allan BJM, Miller GM, McCormick MI, Domenici P, Munday PL. 2014. Parental effects improve escape performance of juvenile reef fish in a high-CO_2 world. Proc R Soc B. 281:20132179.

Allgeier JE, Valdivia A, Cox C, Layman CA. 2016. Fishing down nutrients on coral reefs. Nat Commun. 7:12461.

Allison EH, Mvula PM. 2002. Fishing Livelihoods and Fisheries Management in Malawi. LADDER Working Paper No. 22.https://assets.publishing.service.gov.uk/media/57a08d45e5274a31e000176c/Ladderwp22.pdf (accessed 23 June 2017).

Amorim MCP, Knight ME, Stratoudakis Y, Turner GF. 2004. Differences in sounds made by courting males of three closely related Lake Malawi cichlid species. J Fish Biol. 65:1358–1371.

Amorim MCP, Simões JM, Fonseca PJ, Turner GF. 2008. Species differences in courtship acoustic signals among five Lake Malawi cichlid species (*Pseudotropheus* spp.). J Fish Biol. 72:1355–1368.

Amorim MCP. 1996. Sound production in the blue-green damselfish, *Chromis viridis* (Cuvier, 1830) (Pomacentridae). Bioacoustics. 6:265–272.

Anderson PA, Berzins IK, Fogarty F, Hamlin HJ, Guillette LJ. 2011. Sound, stress, and seahorses: the consequences of a noisy environment to animal health. Aquaculture. 311:129–138.

Avaliani TV, Klyueva NN, Apraksina NK, Tsikunov SG. 2018. Gender-specific features of behavior and lipid spectrum in rats at a remote period after exposure to vibrational noise. J Evol Biochem Physiol. 54:123–129.

Bailey H, Senior B, Simmons D, Rusin J, Picken G. 2010. Assessing underwater noise levels during pile-driving at an offshore windfarm and its potential effects on marine mammals. Mar Pollut Bull. 60:888–897.

Banner A, Hyatt M. 1973. Effects of noise on eggs and larvae of two estuarine fishes. Trans Am Fish Soc. 102:134–136.

Bart AN, Clark J, Young J, Zohar Y. 2001. Underwater ambient noise measurements in aquaculture systems: a survey. Aquac Eng. 25:99–110.

Barton BA, Ribas L, Acerete L, Tort L. 2005. Effects of chronic confinement on physiological responses of juvenile gilthead sea bream, *Sparus aurata L.*, to acute handling. Aquac Res. 36:172–179.

Barton BA. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integr Comp Biol. 42:517–525.

Be Whale Wise. 2019. Marine Wildlife Guidelines. (accessed 2019 Apr 12). http://www.bewhalewise.org/marine-wildlife-guidelines/.

Bejder L, Samuels A, Whitehead H, Finn H, Allen S. 2009. Impact assessment research: use and misuse of habituation, sensitisation and tolerance in describing wildlife responses to anthropogenic stimuli. Mar Ecol Prog Ser. 395:177–185.

Bertucci F, Attia J, Beauchaud M, Mathevon N. 2012. Sounds produced by the cichlid fish *Metriaclima zebra* allow reliable estimation of size and provide information on individual identity. J Fish Biol. 80:752–766.

Bertucci F, Beauchaud M, Attia J, Mathevon N. 2010. Sounds modulate males' aggressiveness in a cichlid fish. Ethology. 116:1179–1188.

Beukers JS, Jones GP. 1997. Habitat complexity modifies the impact of piscivores on a coral reef fish population. Oecologia. 114:50–59.

Bijlsma R, Loeschcke V. 2012. Genetic erosion impedes adaptive responses to stressful environments. Evol Appl. 5:117–129.

Biro PA, Booth DJ. 2009. Extreme boldness precedes starvation mortality in six-lined trumpeter (*Pelates sexlineatus*). Hydrobiologia. 635:395–398.

Bittencourt L, Carvalho RR, Lailson-Brito J, Azevedo AF. 2014. Underwater noise pollution in a coastal tropical environment. Mar Pollut Bull. 83:331–336.

Bittencourt L, Lima IMS, Andrade LG, Carvalho RR, Bisi TL, Lailson-Brito J, Azevedo AF. 2017. Underwater noise in an impacted environment can affect Guiana dolphin communication. Mar Pollut Bull. 114:1130–1134.

Blanchette A, Ely T, Zeko A, Sura SA, Turba R, Fong P. 2019. Damselfish *Stegastes nigricans* increase algal growth within their territories on shallow coral reefs via enhanced nutrient supplies. J Exp Mar Bio Ecol. 513:21–26.

Blickley JL, Blackwood D, Patricelli GL. 2012. Experimental evidence for the effects of chronic anthropogenic noise on abundance of greater sage-grouse at leks. Conserv Biol. 26:461–471.

Bolle LJ, de Jong CAF, Bierman SM, van Beek PJG, van Keeken OA, Wessels PW, van Damme CJG, Winter HV, de Haan D, Dekeling RPA. 2012. Common sole larvae survive high levels of pile-driving sound in controlled exposure experiments. PLoS One. 7:e33052.

Bolnick DI, Amarasekare P, Araújo MS, Bürger R, Levine JM, Novak M, Rudolf VHW, Schreiber SJ, Urban MC, Vasseur D. 2011. Why intraspecific trait variation matters in community ecology. Trends Ecol Evol. 26:183–192.

Bolnick DI, Svanbäck R, Fordyce JA, Yang LH, Davis JM, Hulsey CD. 2003. The ecology of individuals: incidence and implications of individual specialization. Am Nat. 161:1–28.

Bonaldo RM, Bellwood DR. 2008. Size-dependent variation in the functional role of the parrotfish *Scarus rivulatus* on the Great Barrier Reef, Australia. Mar Ecol Prog Ser. 360:237–244.

Borenstein M. 2009. Effect sizes for continuous data. In Copper H, Hedges LV, Valentine JC, editors. The Handbook of Research Synthesis & Meta-Analysis. New York: Russell Sage Foundation. p. 222–235.

Borenstein M, Hedges LV, Higgins JPT, Rothstein HR. 2009. Complex data structures. In Sharples K, editor. Introduction to Meta-Analysis. Chester, England: Wiley. p.217–223.

Boström-Einarsson L, Bonin MC, Munday PL, Jones GP. 2018. Loss of live coral compromises predator-avoidance behaviour in coral reef damselfish. Sci Rep. 8:7795.

Branstetter BK, Bowman VF, Houser DS, Tormey M, Banks P, Finneran JJ, Jenkins K. 2018. Effects of vibratory pile driver noise on echolocation and vigilance in bottlenose dolphins (*Tursiops truncatus*). J Acoust Soc Am. 143:429–439.

Brooker A, Humphrey V. 2016. Measurement of radiated underwater noise from a small research vessel in shallow water. Ocean Eng. 120:182–189.

Brosset P, Ménard F, Fromentin J-M, Bonhommeau S, Ulses C, Bourdeix J-H, Bigot J-L, Van Beveren E, Roos D, Saraux C. 2015. Influnce of environmental variability and age on the body condition of small pelagic fish in the Gulf of Lions. Mar Ecol Prog Ser. 529:219–231.

Brown MR, Fisher LA, Rivier J, Spiess J, Rivier C, Vale W. 1982. Corticotropin-releasing factor: effects on the sympathetic nervous system and oxygen consumption. Life Sci. 30:207–210.

Brumm H, Slabbekoorn H. 2005. Acoustic communication in noise. Adv Study Behav. 35:151–209.

Bruintjes R, Radford AN. 2014. Chronic playback of boat noise does not impact hatching success or post-hatching larval growth and survival in a cichlid fish. PeerJ. 2:e594.

Bruintjes R, Radford AN. 2013. Context-dependent impacts of anthropogenic noise on individual and social behaviour in a cooperatively breeding fish. Anim Behav. 85:1343–1349.

Bruintjes R, Simpson SD, Harding H, Bunce T, Benson T, Rossington K, Jones D. 2016a. The impact of experimental impact pile driving on oxygen uptake in black seabream and plaice. Proc Meet Acoust. 27:010042 .

Bruintjes R, Purser J, Everley KA, Mangan S, Simpson SD, Radford AN. 2016b. Rapid recovery following short-term acoustic disturbance in two fish species. R Soc open Sci. 3:150686 .

Bui S, Oppedal F, Korsøen Ø, Sonny D, Dempster T. 2013. Group behavioural responses of Atlantic salmon (*Salmo salar L.*) to light, infrasound and sound stimuli. PLoS One. 8:e63696.

Burger J, Gochfeld M. 1999. Role of human disturbance in response behavior of laysan albatrosses (*Diomedea immutabilis*). Bird Behav. 13:22–30.

Buxton RT, McKenna MF, Mennitt D, Fristrup K, Crooks K, Angeloni L, Wittemyer G. 2017. Noise pollution is pervasive in U.S. protected areas. Science. 356:531–533.

Caraco T, Blanckenhorn WU, Gregory GM, Newman JA, Recer GM, Zwicker SM. 1990. Risk-sensitivity: ambient temperature affects foraging choice. Anim Behav. 39:338–345.

Carere C, Caramaschi D, Fawcett TW. 2010. Covariation between personalities and individual differences in coping with stress: converging evidence and hypotheses. Curr Zool. 56:728–741.

Casasole G, Raap T, Costantini D, AbdElgawad H, Asard H, Pinxten R, Eens M. 2017. Neither artificial light at night, anthropogenic noise nor distance from roads are associated with oxidative status of nestlings in an urban population of songbirds. Comp Biochem Physiol Part A. 210:14–21.

Casper BM, Halvorsen MB, Matthews F, Carlson TJ, Popper AN. 2013. Recovery of barotrauma injuries resulting from exposure to pile driving sound in two sizes of hybrid striped bass. PLoS One. 8:e73844.

Ceccarelli DM, Jones GP, McCook LJ. 2001. Territorial damselfish as determinants of the structure of benthic communities on coral reef. Oceanogr Mar Biol. 39:355–389.

Ceccarelli DM. 2007. Modification of benthic communities by territorial damselfish: a multi-species comparison. Coral Reefs. 26:853–866.

Chalfoun AD, Thompson FR, Ratnaswamy MJ. 2002. Nest predators and fragmentation: a review and meta-analysis. Conserv Biol. 16:306–318.

Chan AAY-H, Giraldo-Perez P, Smith S, Blumstein DT. 2010. Anthropogenic noise affects risk assessment and attention: the distracted prey hypothesis. Biol Lett. 6:458–461.

Chan AAY-H, Blumstein DT. 2011. Attention, noise, and implications for wildlife conservation and management. Appl Anim Behav Sci. 131:1–7.

Chapman C, Hawkins A. 1973. A field study of hearing in the cod, *Gadus morhua L*. J Comp Physiol. 85:147–167.

Charette C, Derry AM. 2016. Climate alters intraspecific variation in copepod effect traits through pond food webs. Ecology. 97:1239–1250.

Chen I-H, Chou L-S, Chou S-J, Wang J-H, Stott J, Blanchard M, Jen I-F, Yang W-C. 2018. Sound exposure-induced cytokine gene transcript profile changes in captive bottlenose dolphin (*Tursiops truncatus*) blood identified by a probe-based qRT-PCR. J Vet Med Sci. 80:601–605.

Chevin L-M, Lande R, Mace GM. 2010. Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. PLoS Biol. 8:e1000357.

Chin A, Lison De Loma T, Reytar K, Planes S, Gerhardt K, Clua E, Burke L, Wilkinson C. 2011. Status of Coral Reefs of the Pacific and Outlook. Global Coral Reef Monitoring Network. p. 1–22.

Cianciaruso MV, Batalha MA, Gaston KJ, Petchey OL. 2009. Including intraspecific variability in functional diversity. Ecology. 90:81–89.

Clarke JA. 1983. Moonlight's influence on predator/prey interactions between short-eared owls (*Asio flammeus*) and deermice (*Peromyscus maniculatus*). Behav Ecol Sociobiol. 13:205–209.

Clarke, KR. 1993. Non-parametric multivariate analyses of changes in community structure. Aust J Ecol. 18:117–143.

Clarke KR, Warwick RM. 2001. Change in marine communities: an approach to statistical analysis and interpretation. 2nd ed. Plymouth, UK: Primer-E Ltd.

Coker DJ, Graham NAJ, Pratchett MS. 2012a. Interactive effects of live coral and structural complexity on the recruitment of reef fishes. Coral Reefs. 31:919–927.

Coker DJ, Pratchett MS, Munday PL. 2012b. Influence of coral bleaching, coral mortality and conspecific aggression on movement and distribution of coral-dwelling fish. J Exp Mar Bio Ecol. 414–415:62–68.

Coker DJ, Pratchett MS, Munday PL. 2009. Coral bleaching and habitat degradation increase susceptibility to predation for coral-dwelling fishes. Behav Ecol. 20:1204–1210.

Colleye O, Kéver L, Lecchini D, Berten L, Parmentier E. 2016. Auditory evoked potential audiograms in post-settlement stage individuals of coral reef fishes. J Exp Mar Bio Ecol. 483:1–9.

Connon RE, Jeffries KM, Komoroske LM, Todgham AE, Fangue NA. 2018. The utility of transcriptomics in fish conservation. J Exp Biol. 221:jeb148833.

Conomy JT, Dubovsky JA, Collazo JA, Fleming WJ. 1998. Do black ducks and wood ducks habituate to aircraft disturbance? J Wildl Manage. 62:1135–1142.

Cook KV, O'Connor CM, McConnachie SH, Gilmour KM, Cooke SJ. 2012. Condition dependent intra-individual repeatability of stress-induced cortisol in a freshwater fish. Comp Biochem Physiol - A Mol Integr Physiol. 161:337–343.

Correa JMG, Sempere JTB, Juanes F, Rountree R, Ruíz JF, Ramis J. 2019. Recreational boat traffic effects on fish assemblages: first evidence of detrimental consequences at regulated mooring zones in sensitive marine areas detected by passive acoustics. Ocean Coast Manag. 168:22–34.

Côté IM, Darling ES, Brown CJ. 2016. Interactions among ecosystem stressors and their importance in conservation. Proc R Soc B. 283:20152592.

Cox K, Brennan LP, Gerwing TG, Dudas SE, Juanes F. 2018. Sound the alarm: a meta-analysis on the effect of aquatic noise on fish behavior and physiology. Glob Chang Biol. 24:3105–3116.

Cripps G, Lindeque P, Flynn KJ. 2014. Have we been underestimating the effects of ocean

acidification in zooplankton? Glob Chang Biol. 20:3377–3385.

Crutsinger GM, Collins MD, Fordyce JA, Gompert Z, Nice CC, Sanders NJ. 2006 U. 2006. Plant genotypic diversity predicts community structure and governs an ecosystem process. Science. 313:966–968.

Da Silva A, Samplonius JM, Schlicht E, Valcu M, Kempenaers B. 2014. Artificial night lighting rather than traffic noise affects the daily timing of dawn and dusk singing in common European songbirds. Behav Ecol. 25:1037–1047.

Dall SRX, Houston AI, McNamara JM. 2004. The behavioural ecology of personality: consistent individual differences from an adaptive perspective. Ecol Lett. 7:734–739.

Dalla C, Pitychoutis PM, Kokras N, Papadopoulou-Daifoti Z. 2011. Sex differences in response to stress and expression of depressive-like behaviours in the rat. In Neill JC, Kulkarni J, editors. Biological Basis of Sex Differences in Psychopharmacology. Heidelberg, Berlin: Springer-Verlag. p. 97–118.

Darling ES, Côté IM. 2008. Quantifying the evidence for ecological synergies. Ecol Lett. 11:1278–1286.

Davenport J, Davenport JL. 2006. The impact of tourism and personal leisure transport on coastal environments: a review. Estuar Coast Shelf Sci. 67:280–292.

Davidson J, Bebak J, Mazik P. 2009. The effects of aquaculture production noise on the growth, condition factor, feed conversion, and survival of rainbow trout, *Oncorhynchus mykiss*. Aquaculture. 288:337–343.

Davidson J, Frankel AS, Ellison WT, Summerfelt S, Popper AN, Mazik P, Bebak J. 2007. Minimizing noise in fiberglass aquaculture tanks: noise reduction potential of various retrofits. Aquac Eng. 37:125–131.

Davies S, Haddad N, Ouyang JQ. 2017. Stressful city sounds: glucocorticoid responses to experimental traffic noise are environmentally dependent. Biol Lett. 13:20170276.

Debusschere E, Hostens K, Adriaens D, Ampe B, Botteldooren D, De Boeck G, De Muynck A, Sinha AK, Vandendriessche S, Van Hoorebeke L, et al. 2016. Acoustic stress responses in juvenile sea bass *Dicentrarchus labrax* induced by offshore pile driving. Environ Pollut. 208:747–757.

Des Roches S, Post DM, Turley NE, Bailey JK, Hendry AP, Kinnison MT, Schweitzer JA, Palkovacs EP. 2017. The ecological importance of intraspecific variation. Nat Ecol Evol. 2:57–64.

Dias BG, Ressler KJ. 2014. Parental olfactory experience influences behavior and neural structure in subsequent generations. Nat Neurosci. 17:89–96.

Díaz S, Lavorel S, Chapin III FS, Tecco PA, Gurvich DE, Grigulis K. 2007. Functional diversity – at the crossroads between ecosystem functioning and environmental filter. In: Canadell JG, Pataki D, Pitelka L, editors. Terrestrial Ecosystems in a Changing World. Heidelberg, Berlin: Springer-Verlag. p. 81–90.

Dinh JP, Suca JJ, Lillis A, Apprill A, Llopiz JK, Mooney TA. 2018. Multiscale spatio-temporal patterns of boat noise on U.S. Virgin Island coral reefs. Mar Pollut Bull. 136:282–290.

Dissanayake A, Galloway TS, Jones MB. 2008. Nutritional status of *Carcinus maenas* (Crustacea: Decapoda) influences susceptibility to contaminant exposure. Aquat Toxicol. 89:40–46.

Dorado-Correa AM, Rodriguez-Rocha M, Brumm H. 2016. Anthropogenic noise, but not artificial light levels predicts song behaviour in an equatorial bird. R Soc open Sci. 3:160231.

Dorado-Correa AM, Zollinger SA, Heidinger B, Brumm H. 2018. Timing matters: traffic noise accelerates telomere loss rate differently across developmental stages. Front Zool. 15:29.

Doyon C, Trudeau VL, Moon TW. 2005. Stress elevates corticotropin-releasing factor (CRF) and CRF-binding protein mRNA levels in rainbow trout (*Oncorhynchus mykiss*). J Endocrinol. 186:123–130.

Drevon D, Fursa SR, Malcolm AL. 2017. Intercoder reliability and validity of webplotdigitizer in extracting graphed data. Behav Modif. 41:323–339.

Duckworth RA, Mendonca MT, Hill GE. 2001. A condition dependent link between testosterone and disease resistance in the house finch. Proc R Soc B. 268:2467–2472.

Dutil J-D, Lambert Y. 2011. Natural mortality from poor condition in Atlantic cod (*Gadus morhua*). Can J Fish Aquat Sci. 57:826–836.

Ebrahimi A, Razaghian AH, Seif MS, Zahedi F, Nouri-Borujerdi A. 2019. A comprehensive study on noise reduction methods of marine propellers and design procedures. Appl Acoust. 150:55–69.

Ellison WT, Southall BL, Clark CW, Frankel AS. 2012. A new context-based approach to assess marine mammal behavioral responses to anthropogenic sounds. Conserv Biol. 26:21–28.

Engås A, Løkkeborg S, Ona E, Soldal AV. 1996. Effects of seismic shooting on local abundance and catch rates of cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*). Can J Fish Aquat Sci. 53:2238–2249.

Erbe C, Williams R, Sandilands D, Ashe E. 2014. Identifying modeled ship noise hotspots for marine mammals of Canada's Pacific region. PLoS One. 9:e89820.

Erbe C. 2013. Underwater noise of small personal watercraft (jet skis). J Acoust Soc Am. 133:EL326–EL330.

FAO. 2018. The State of World Fisheries and Aquaculture 2018. www.fao.org/publications. (accessed

2019 May 10).

Farcas A, Thompson PM, Merchant ND. 2016. Underwater noise modelling for environmental impact assessment. Environ Impact Assess Rev. 57:114–122.

Fasola E, Ribeiro R, Lopes I. 2015. Microevolution due to pollution in amphibians: a review on the genetic erosion hypothesis. Environ Pollut. 204:181–190.

Faurea G. 1989. Degradation of coral reefs at Moorea Island (French Polynesia) by *Acanthaster planci*. J Coast Res. 5:295–305.

Fay RR, Megela-Simmons A. 1999. The sense of hearing in fishes and amphibians. In Fay RR, Popper AN, editors. Comparative hearing: fish and amphibians. New York: Springer Verlag. p. 269–318.

Feary DA, Almany GR, Mccormick MI, Jones GP. 2007. Habitat choice, recruitment and the response of coral reef fishes to coral degradation. Oecologia. 153:727–737.

Feary DA, McCormick MI, Jones GP. 2009. Growth of reef fishes in response to live coral cover. J Exp Mar Bio Ecol. 373:45–49.

Ferrari MCO, McCormick MI, Meekan MG, Simpson SD, Nedelec SL, Chivers DP. 2018. School is out on noisy reefs: the effect of boat noise on predator learning and survival of juvenile coral reef fishes. Proc R Soc B. 285:20180033.

Filiciotto F, Vazzana M, Celi M, Maccarrone V, Ceraulo M, Buffa G, Di Stefano V, Mazzola S, Buscaino G. 2014. Behavioural and biochemical stress responses of *Palinurus elephas* after exposure to boat noise pollution in tank. Mar Pollut Bull. 84:104–114.

FishBase. 2017. Froese R, Pauly D, editors. www.fishbase.org. (accessed 2017 Jul 28).

Fitzgibbon QP, Day RD, McCauley RD, Simon CJ, Semmens JM. 2017. The impact of seismic air gun exposure on the haemolymph physiology and nutritional condition condition of spiny lobster, *Jasus edwardsii*. Mar Pollut Bull. 125:146–156.

Francis CD, Ortega CP, Cruz A. 2009. Noise pollution changes avian communities and species interactions. Curr Biol. 19:1415–1419.

Francis CD, Barber JR. 2013. A framework for understanding noise impacts on wildlife: an urgent conservation priority. Front Ecol Environ. 11:305–313.

Francis CD, Kleist NJ, Ortega CP, Cruz A. 2012. Noise pollution alters ecological services: enhanced pollination and disrupted seed dispersal. Proc R Soc B. 279:2727–2735.

Frisk G V. 2012. Noiseonomics: the relationship between ambient noise levels in the sea and global economic trends. Sci Rep. 2:437.

Froukh T, Kochzius M. 2008. Species boundaries and evolutionary lineages in the blue green damselfishes *Chromis viridis* and *Chromis atripectoralis* (Pomacentridae). J Fish Biol. 72:451–457.

Gardner RW, Smith LW, Park RL. 1988. Feeding and management of dairy heifers for optimal lifetime productivity. J Dairy Sci. 71:996–999.

Gentry KE, Derryberry EP, Danner RM, Danner JE, Luther DA. 2017. Immediate signaling flexibility in response to experimental noise in urban, but not rural, white-crowned sparrows. Ecosphere. 8:e01916.

Gerlai R. 2007. Mate choice and hybridization in Lake Malawi cichlids, *Sciaenochromis fryeri* and *Cynotilapia afra*. Ethology. 113:673–685.

Gillett R, Wayne M. 2006. Spearfishing in the Pacific Islands: current status and management issues. Rome: Food and Agricultural Organisation of the United Nations. p.72.

Goldbogen JA, Southall BL, DeRuiter SL, Calambokidis J, Friedlaender AS, Hazen EL, Falcone EA, Schorr GS, Douglas A, Moretti DJ, et al. 2013. Blue whales respond to simulated mid-frequency military sonar. Proc R Soc B. 280:20130657.

Gonson C, Pelletier D, Alban F, Giraud-Carrier C, Ferraris J. 2017. Influence of settings management and protection status on recreational uses and pressures in marine protected areas. J Environ Manage. 200:170–185.

Gonson C, Pelletier D, Gamp E, Preuss B, Jollit I, Ferraris J. 2016. Decadal increase in the number of recreational users is concentrated in no-take marine reserves. Mar Pollut Bull. 107:144–154.

Gordon TAC, Cowburn B, Sluka RD. 2015. Defended territories of an aggressive damselfish contain lower juvenile coral density than adjacent non-defended areas on Kenyan lagoon patch reefs. Coral Reefs. 34:13–16.

Gordon TAC, Harding HR, Wong KE, Merchant ND, Meekan MG, McCormick MI, Radford AN, Simpson SD. 2018. Habitat degradation negatively affects auditory settlement behavior of coral reef fishes. Proc Natl Acad Sci USA. 115:5193–5198.

Grabarczyk EE, Pipkin MA, Vonhof MJ, Gill SA. 2018. When to change your tune? Unpaired and paired male house wrens respond differently to anthropogenic noise. J Ecoacoust. 2:#LHGRVC.

Graham NAJ, Wilson SK, Pratchett MS, Polunin NVC, Spalding MD. 2009. Coral mortality versus structural collapse as drivers of corallivorous butterflyfish decline. Biodivers Conserv. 18:3325–3336.

Great Barrier Reef Marine Park Authority. 2014. Great Barrier Reef Outlook Report 2014. GBRMPA, Townsville.

Griffen BD, Norelli AP. 2015. Spatially variable habitat quality contributes to within-population variation in reproductive success. Ecol Evol. 5:1474–1483.

Grüebler MU, Schuler H, Müller M, Spaar R, Horch P, Naef-Daenzer B. 2008. Female biased mortality caused by anthropogenic nest loss contributes to population decline and adult sex ratio of a

meadow bird. Biol Conserv. 141:3040–3049.

Guillette LM, Reddon AR, Hoeschele M, Sturdy CB. 2011. Sometimes slower is better: slow-exploring birds are more sensitive to changes in a vocal discrimination task. Proc R Soc B. 278:767–773.

Gunderson AR, Armstrong EJ, Stillman JH. 2016. Multiple stressors in a changing world: the need for an improved perspective on physiological responses to the dynamic marine environment. Ann Rev Mar Sci. 8:357–378.

Gurule-Small GA, Tinghitella RM. 2018. Developmental experience with anthropogenic noise hinders adult mate location in an acoustically signalling invertebrate. Biol Lett. 14:20170714.

Habib L, Bayne EM, Boutin S. 2006. Chronic industrial noise affects pairing success and age structure of ovenbirds *Seiurus aurocapilla*. J Appl Ecol. 44:176–184.

Halvorsen M, Casper B, Woodley C, Carlson T. 2012. Threshold for onset of injury in Chinook salmon from exposure to impulsive pile driving sounds. PLoS One. 7:e38968.

Halvorsen M, Wysocki L, Stehr C. 2009. Barging effects on sensory systems of Chinook salmon smolts. Trans Am Fish Soc. 138:777–789.

Handegard NO, De Robertis A, Rieucau G, Boswell K, Macaulay GJ. 2015. The reaction of a captive herring school to playbacks of a noise-reduced and a conventional research vessel. Can J Fish Aquat Sci. 72:491–499.

Harding HR, Gordon TAC, Eastcott E, Simpson SD, Radford AN. 2019. Causes and consequences of intraspecific variation in animal responses to anthropogenic noise. Behav Ecol. Online early.

Harding HR, Gordon TAC, Hsuan RE, Mackaness ACE, Radford AN, Simpson SD. 2018. Fish in habitats with higher motorboat disturbance show reduced sensitivity to motorboat noise. Biol Lett. 14:20180441.

Harris CM, Thomas L, Falcone EA, Hildebrand J, Houser D, Kvadsheim PH, Lam F-PA, O Miller PJ, Moretti DJ, Read AJ, et al. 2018. Marine mammals and sonar: dose-response studies, the risk-disturbance hypothesis and the role of exposure context. J Appl Ecol. 55:396–404.

Hartig F. 2017. DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. https://CRAN.R-project.org/package=DHARMa.

Hata H, Kato M. 2002. Weeding by the herbivorous damselfish *Stegastes nigricans* in nearly monocultural algae farms. Mar Ecol Prog Ser. 237:227–231.

Hata H, Umezawa Y. 2011. Food habits of the farmer damselfish *Stegastes nigricans* inferred by stomach content, stable isotope, and fatty acid composition analyses. Ecol Res. 26:809–818.

Haver SM, Gedamke J, Hatch LT, Dziak RP, Van Parijs S, McKenna MF, Barlow J, Berchok C, DiDonato E, Hanson B, et al. 2018. Monitoring long-term soundscape trends in U.S. Waters: the NOAA/NPS

Ocean Noise Reference Station Network. Mar Policy. 90:6–13.

Hawkins A, Johnstone A. 1978. The hearing of the Atlantic salmon, *Salmo salar*. J Fish Biol. 13:655–673.

Hawkins AD, Popper AN. 2016. A sound approach to assessing the impact of underwater noise on marine fishes and invertebrates. ICES J Mar Sci. 74:635–651.

Hawkins AD, Pembroke AE, Popper AN. 2015. Information gaps in understanding the effects of noise on fishes and invertebrates. Rev Fish Biol Fisheries. 25:39–64.

Hawkins AD, Roberts L, Cheesman S. 2014. Responses of free-living coastal pelagic fish to impulsive sounds. J Acoust Soc Am. 135:3101–3116.

Heiler J, Elwen SH, Kriesell HJ, Gridley T. 2016. Changes in bottlenose dolphin whistle parameters related to vessel presence, surface behaviour and group composition. Anim Behav. 117:167–177.

Herbert N, Steffensen J. 2005. The response of Atlantic cod, *Gadus morhua*, to progressive hypoxia: fish swimming speed and physiological stress. Mar Biol. 147:1403–1412.

Herbert-Read JE, Kremer L, Bruintjes R, Radford AN, Ioannou CC. 2017. Anthropogenic noise pollution from pile-driving disrupts the structure and dynamics of fish shoals. Proc R Soc B. 284:20171627.

Herrera-Montes MI, Aide TM. 2011. Impacts of traffic noise on anuran and bird communities. Urban Ecosyst. 14:415–427.

Hert E. 1992. Homing and home-site fidelity in rock-dwelling cichlids (Pisces: Teleostei) of Lake Malawi, Africa. Environ Biol Fishes. 33:229–237.

Hert E. 1995. The impact of intralacustrine introductions with regard to space utilization and competition for territories to a cichlid fish community in Lake Malawi , Africa. Ecol Res. 10:117–124.

Hewitt CL, Willing J, Bauckham A, Cassidy AM, Cox CMS, Jones L, Wotton DM. 2004. New Zealand marine biosecurity: delivering outcomes in a fluid environment. New Zeal J Mar Fresh. 38:429–438.

Hildebrand J. 2005. Impacts of anthropogenic sound. In: Reynolds III JE, Perrin WF, Reeves RR, Montgomery S, Ragen TJ, editors. Marine Mammal Research: Conservation Beyond Crisis. Baltimore, Maryland: The John Hopkins University Press. p. 101–123.

Hildebrand JA. 2009. Anthropogenic and natural sources of ambient noise in the ocean. Mar Ecol Prog Ser. 395:5–20.

Höglund E, Gjøen H-M, Pottinger TG, Øverli Ø. 2008. Parental stress-coping styles affect the behaviour of rainbow trout *Oncorhynchus mykiss* at early developmental stages. J Fish Biol.

73:1764–1769.

Holles SH, Simpson SD, Radford AN, Berten L, Lecchini D. 2013. Boat noise disrupts orientation behaviour in a coral reef fish. Mar Ecol Prog Ser. 485:295–300.

Holmes LJ, McWilliam J, Ferrari MCO, McCormick MI. 2017. Juvenile damselfish are affected but desensitize to small motor boat noise. J Exp Mar Bio Ecol. 494:63–68.

Holmlund CM, Hammer M. 1999. Ecosystem services generated by fish populations. Ecol Econ. 29:253–268.

Holt DE, Johnston CE. 2011. Can you hear the dinner bell? Response of cyprinid fishes to environmental acoustic cues. Anim Behav. 82:529–534.

Homan RN, Regosin J V, Rodrigues DM, Reed JM, Windmiller BS, Romero LM. 2003. Impacts of varying habitat quality on the physiological stress of spotted salamanders (*Ambystoma maculatum*). Anim Conserv. 6:11–18.

Houser DS, Martin SW, Finneran JJ. 2013a. Behavioral responses of California sea lions to mid-frequency (3250-3450 Hz) sonar signals. Mar Environ Res. 92:268–278.

Houser DS, Martin SW, Finneran JJ. 2013b. Exposure amplitude and repetition affect bottlenose dolphin behavioral responses to simulated mid-frequency sonar signals. J Exp Mar Bio Ecol. 443:123–133.

Hughes TP, Bellwood DR, Folke C, Steneck RS, Wilson J. 2005. New paradigms for supporting the resilience of marine ecosystems. Trends Ecol Evol. 20:380–386.

Hughes TP, Kerry J, Álvarez-Noriega M, Álvarez-Romero J, Anderson K, Baird A, Babcock R, Beger M, Bellwood D, Berkelmans R, et al. 2017. Global warming and recurrent mass bleaching of corals. Nature. 543:373–377.

Husté A, Clobert J, Miaud C. 2006. The movements and breeding site fidelity of the natterjack toad (*Bufo calamita*) in an urban park near Paris (France) with management recommendations. Amphib Reptil. 27:561–568.

Illing B, Rummer JL. 2017. Physiology can contribute to better understanding, management, and conservation of coral reef fishes. Conserv Physiol. 5:cox005.

Injaian AS, Poon LY, Patricelli GL. 2018. Effects of experimental anthropogenic noise on avian settlement patterns and reproductive success. Behav Ecol. 29:1181–1189.

Jacobsen L, Baktoft H, Jepsen N, Aarestrup K, Berg S, Skov C. 2014. Effect of boat noise and angling on lake fish behaviour. J Fish Biol. 84:1768–1780.

Jain-Schlaepfer S, Fakan E, Rummer JL, Simpson SD, McCormick MI. 2018. Impact of motorboats on fish embryos depends on engine type. Conserv Physiol. 6:coy014.

Jensen MP, Allen CD, Eguchi T, Bell IP, LaCasella EL, Hilton WA, Hof CAM, Dutton PH. 2018. Environmental warming and feminization of one of the largest sea turtle populations in the world. Curr Biol. 28:154–159.

Job JR, Kohler SL, Gill SA. 2016. Song adjustments by an open habitat bird to anthropogenic noise, urban structure, and vegetation. Behav Ecol. 27:1734–1744.

Johnston M, Dawson J, De Souza E, Stewart EJ. 2017. Management challenges for the fastest growing marine shipping sector in Arctic Canada: pleasure crafts. Polar Rec. 53:67–78.

Karino K, Nakazono A. 1993. Reproductive behavior of the territorial herbivore *Stegastes nigricans* (Pisces: Pomacentridae) in relation to colony formation. J Ethol. 11:99–110.

Kastelein RA, Jennings N, Kommeren A, Helder-Hoek L, Schop J. 2017. Acoustic dose-behavioral response relationship in sea bass (*Dicentrarchus labrax*) exposed to playbacks of pile driving sounds. Mar Environ Res. 130:315–324.

Kenyon T, Ladich F, Yan H. 1998. A comparative study of hearing ability in fishes: the auditory brainstem response approach. J Comp Physiol A. 182:307–318.

Kenyon TN. 1996. Ontogenetic changes in the auditory sensitivity of damselfishes (Pomacentridae). J Comp Physiol A. 179:553–561.

Kiffney PM, Clements WH. 1996. Size-dependent response of macroinvertebrates to metals in experimental streams. Environ Toxicol Chem. 15:1352–1356.

Kight CR, Swaddle JP. 2011. How and why environmental noise impacts animals: an integrative, mechanistic review. Ecol Lett. 14:1052–1061.

Killen SS, Marras S, Mckenzie DJ. 2011. Fuel, fasting, fear: routine metabolic rate and food deprivation exert synergistic effects on risk-taking in individual juvenile European sea bass. J Anim Ecol. 80:1024–1033.

King GD, Chapman JM, Cooke SJ, Suski CD. 2016. Stress in the neighborhood: tissue glucocorticoids relative to stream quality for five species of fish. Sci Total Environ. 547:87–94.

Klumpp DW, Mckinnon D, Daniel P. 1987. Damselfish territories: zones of high productivity on coral reefs. Mar Ecol Prog Ser. 40:41–51.

Knudsen F, Enger P, Sand O. 1992. Awareness reactions and avoidance responses to sound in juvenile Atlantic salmon, *Salmo salar L*. J Fish Biol. 40:523–534.

Koenigstein S, Mark FC, Gößling-Reisemann S, Reuter H, Poertner H-O. 2016. Modelling climate change impacts on marine fish populations: process-based integration of ocean warming, acidification and other environmental drivers. Fish Fish. 17:972–1004.

Konings A. 2001. Malawi cichlids in their natural habitat. Cichlid Press. El Paso, Texas.

Koops MA, Hutchings JA, McIntyre TM. 2004. Testing hypotheses about fecundity, body size and maternal condition in fishes. Fish Fish. 5:120–130.

Kroodsma DE, Byers BE, Goodale E, Johnson S, Liu WC. 2001. Pseudoreplication in playback experiments, revisited a decade later. Anim Behav. 61:1029–1033.

Kunc HP, McLaughlin KE, Schmidt R. 2016. Aquatic noise pollution: implications for individuals, populations, and ecosystems. Proc R Soc B. 283:20160839.

Kuningas S, Kvadsheim PH, Lam F-PA, Miller PJO. 2013. Killer whale presence in relation to naval sonar activity and prey abundance in northern Norway. ICES J Mar Sci. 70:1287–1293.

Ladich F, Schulz-mirbach T. 2013. Hearing in cichlid fishes under noise conditions. PLoS One. 8:e57588.

Ladich F. 2000. Acoustic communication and the evolution of hearing in fishes. Phil Trans R Soc B. 355:1285–1288.

Lam VWY, Cheung WWL, Reygondeau G, Rashid Sumaila U. 2016. Projected change in global fisheries revenues under climate change. Sci Rep. 6:32607.

Lawrence MJ, Zolderdo AJ, Godin J-GJ, Mandelman JW, Gilmour KM, Cooke SJ. 2019. Cortisol does not increase risk of mortality to predation in juvenile bluegill sunfish: a manipulative experimental field study. J Exp Zool Part A Ecol Integr Physiol. 331:253–261.

LaZerte SE, Otter KA, Slabbekoorn H. 2017. Mountain chickadees adjust songs, calls and chorus composition with increasing ambient and experimental anthropogenic noise. Urban Ecosyst. 20:989–1000.

LaZerte SE, Slabbekoorn H, Otter KA. 2016. Learning to cope: vocal adjustment to urban noise is correlated with prior experience in black-capped chickadees. Proc R Soc B. 283:20161058.

Lecchini D, Galzin R. 2005. Spatial repartition and ontogenetic shifts in habitat use by coral reef fishes (Moorea, French Polynesia). Mar Biol. 147:47–58.

Lee CS, Ahn BK, Han JM, Kim JH. 2018. Propeller tip vortex cavitation control and induced noise suppression by water injection. J Mar Sci Technol. 23:453–463.

Lengagne T. 2008. Traffic noise affects communication behaviour in a breeding anuran, *Hyla arborea*. Biol Conserv. 141:2023–2031.

Lenth R. 2018. emmeans: estimated marginal means, aka least-squares means. https://CRAN.R-project.org/package=emmeans.

Letourneur Y, Galzin R, Harmelin-Vivien M. 1997. Temporal variations in the diet of the damselfish *Stegastes nigricans* (Lacepede) on a Reunion fringing reef. J Exp Mar Bio Ecol. 217:1–18.

Lichtenstein JLL, Pruitt JN, Modlmeier AP. 2016. Intraspecific variation in collective behaviors drives interspecific contests in acorn ants. Behav Ecol. 27:553–559.

Lima SL, Dill LM. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Can J Zool. 68:619–640.

Lo-Yat A, Meekan M, Munksgaard N, Parry D, Planes S, Wolter M, Carleton J. 2005. Small-scale spatial variation in the elemental composition of otoliths of *Stegastes nigricans* (Pomacentridae) in French Polynesia. Coral Reefs. 24:646–653.

Long AM, Colon MR, Bosman JL, Robinson DH, Pruett HL, McFarland TM, Mathewson HA, Szewczak JM, Newnam JC, Morrison ML. 2017a. A before-after control-impact assessment to understand the potential impacts of highway construction noise and activity on an endangered songbird. Ecol Evol. 7:379–389.

Long AM, Colon MR, Bosman JL, McFarland TM, Locatelli AJ, Stewart LR, Mathewson HA, Newnam JC, Morrison ML. 2017b. Effects of road construction noise on golden-cheeked warblers: an update. Wildl Soc Bull. 41:240–248.

Lotka AJ. 1925. Elements of physical biology. Sci Prog. 21:341–343.

Lovell JM. 2003. The hearing abilities of the bass, *Dicentrarchus labrax*. Technical report commissioned by ARIA Marine Ltd. for the European Commission Fifth Framework Programme. Project Reference: Q5AW-CT-2001-01896.

Lowry H, Lill A, Wong BBM. 2011. Tolerance of auditory disturbance by an avian urban adapter, the noisy miner. Ethology. 117:490–497.

Luo J, Clarin B-M, Borissov IM, Siemers BM. 2014. Are torpid bats immune to anthropogenic noise? J Exp Biol. 217:1072–1078.

Luther DA, Derryberry EP. 2012. Birdsongs keep pace with city life: changes in song over time in an urban songbird affects communication. Anim Behav. 83:1059–1066.

Lynch AJ, Cooke SJ, Deines AM, Bower SD, Bunnell DB, Cowx IG, Nguyen VM, Nohner J, Phouthavong K, Riley B, et al. 2016. The social, economic, and environmental importance of inland fish and fisheries. Environ Rev. 24:115–121.

Magnhagen C, Johansson K, Sigray P. 2017. Effects of motorboat noise on foraging behaviour in Eurasian perch and roach: a field experiment. Mar Ecol Prog Ser. 564:115–125.

Makochekanwa A. 2013. An analysis of tourism contribution to economic growth in SADC Countries. Botswana J Econ. 11:42–56.

Mallet D, Vigliola L, Wantiez L, Pelletier D. 2016. Diurnal temporal patterns of the diversity and the abundance of reef fishes in a branching coral patch in New Caledonia. Austral Ecol. 41:733–744.

Mancera KF, Lisle A, Allavena R, Phillips CJC. 2017. The effects of mining machinery noise of different

frequencies on the behaviour, faecal corticosterone and tissue morphology of wild mice (*Mus musculus*). Appl Anim Behav Sci. 197:81–89.

Martínez-Mota R, Valdespino C, Sánchez-Ramos MA, Serio-Silva JC. 2007. Effects of forest fragmentation on the physiological stress response of black howler monkeys. Anim Conserv. 10:374–379.

Maruska KP, Ung US, Fernald RD. 2012. The African cichlid fish *Astatotilapia burtoni* uses acoustic communication for reproduction: sound production, hearing, and behavioral significance. PLoS One. 7:e37612.

Maxwell RJ, Zolderdo AJ, de Bruijn R, Brownscombe JW, Staaterman E, Gallagher AJ, Cooke SJ. 2018. Does motor noise from recreational boats alter parental care behaviour of a nesting freshwater fish? Aquat Conserv Freshw Ecosyst. 28:969–978.

McBryan TL, Anttila K, Healy TM, Schulte PM. 2013. Responses to temperature and hypoxia as interacting stressors in fish: implications for adaptation to environmental change. Integr Comp Biol. 53:648–659.

McClellan-Green P, Romano J, Oberdörster E. 2007. Does gender really matter in contaminant exposure? A case study using invertebrate models. Environ Res. 104:183–191.

McClure CJW, Ware HE, Carlisle J, Kaltenecker G, Barber JR. 2013. An experimental investigation into the effects of traffic noise on distributions of birds: avoiding the phantom road. Proc R Soc B. 280:20132290.

McClure CJW, Ware HE, Carlisle JD, Barber JR. 2017. Noise from a phantom road experiment alters the age structure of a community of migrating birds. Anim Conserv. 20:164–172.

McCormick MI, Watson S-A, Simpson SD, Allan BJM. 2018a. Effect of elevated CO_2 and small boat noise on the kinematics of predator–prey interactions. Proc R Soc B. 285:20172650.

McCormick MI, Allan BJM, Harding H, Simpson SD. 2018b. Boat noise impacts risk assessment in a coral reef fish but effects depend on engine type. Sci Rep. 8:3847.

McCormick MI, Fakan EP, Nedelec SL, Allan BJM. 2019. Effects of boat noise on fish fast-start escape response depend on engine type. Sci Rep. 9:6554.

McCormick MI. 2012. Lethal effects of habitat degradation on fishes through changing competitive advantage. Proc R Soc B. 279:3899–3904.

McKenna M, Shannon G, Fristrup K. 2016. Characterizing anthropogenic noise to improve understanding and management of impacts to wildlife. Endanger Species Res. 31:279–291.

McKenna MF, Gabriele C, Kipple B. 2017. Effects of marine vessel management on the underwater acoustic environment of Glacier Bay National Park, AK. Ocean Coast Manag. 139:102–112.

McKenna MF, Wiggins SM, Hildebrand JA. 2013. Relationship between container ship underwater

noise levels and ship design, operational and oceanographic conditions. Sci Rep. 3:1760.

McMahon TA, Rohr JR, Bernal XE. 2017. Light and noise pollution interact to disrupt interspecific interactions. Ecology. 98:1290–1299.

Medina MH, Correa JA, Barata C. 2007. Micro-evolution due to pollution: possible consequences for ecosystem responses to toxic stress. Chemosphere. 67:2105–2114.

Mekonnen MM, Hoekstra AY. 2015. Global gray water footprint and water pollution levels related to anthropogenic nitrogen loads to fresh water. Environ Sci Technol. 49:12860–12868.

Mendl M. 1999. Performing under pressure: stress and cognitive function. Appl Anim Behav Sci. 65:221–244.

Mensinger AF, Putland RL, Radford CA. 2018. The effect of motorboat sound on Australian snapper *Pagrus auratus* inside and outside a marine reserve. Ecol Evol. 8:6438–6448.

Merchant ND, Faulkner RC, Martinez R. 2018. Marine noise budgets in practice. Conserv Lett. 11:e12420.

Merchant ND, Fristrup KM, Johnson MP, Tyack PL, Witt MJ, Blondel P, Parks SE. 2015. Measuring acoustic habitats. Methods Ecol Evol. 6:257–265.

Merchant ND. 2019. Underwater noise abatement: economic factors and policy options. Environ Sci Policy. 92:116–123.

Metcalfe NB, Monaghan P. 2001. Compensation for a bad start: grow now, pay later? Trends Ecol Evol. 16:254–260.

Millennium Ecosystem Assessment. 2005. Ecosystems and human well-being: synthesis. Washington, DC: Island Press.

Mills SC, Beldade R, Chabanet P, Bigot L, O'Donnell JL, Bernardi G. 2015. Ghosts of thermal past: reef fish exposed to historic high temperatures have heightened stress response to further stressors. Coral Reefs. 34:1255–1260.

Mimura M, Yahara T, Faith DP, Vázquez-Domínguez E, Colautti RI, Araki H, Javadi F, Núñez-Farfán J, Mori AS, Zhou S, et al. 2017. Understanding and monitoring the consequences of human impacts on intraspecific variation. Evol Appl. 10:121–139.

Malawi Ministry of Finance & Development. 2012 RAPID: population and development in Malawi.http://www.healthpolicyinitiative.com/Publications/Documents/1065_1_Malawi_booklet_2_23_1_singlepg_acc.pdf. (accessed 23 June 2017).

Moher D, Liberati A, Tetzlaff J, Altman DG. 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA Statement. Ann Intern Med. 151:264–269.

Møller AP. 2008. Flight distance of urban birds, predation, and selection for urban life. Behav Ecol

Sociobiol. 63:63–75.

Moran EV, Hartig F, Bell DM. 2016. Intraspecific trait variation across scales: implications for understanding global change responses. Glob Chang Biol. 22:137–150.

Morgan J, Iwama G. 1996. Cortisol-induced changes in oxygen consumption and ionic regulation in coastal cutthroat trout (*Oncorhynchus clarki clarki*) parr. Fish Physiol Biochem. 15:385–394.

Morley EL, Jones G, Radford AN. 2014. The importance of invertebrates when considering the impacts of anthropogenic noise. Proc R Soc B. 281:20132683.

Mubiana VK, Vercauteren K, Blust R. 2006. The influence of body size, condition index and tidal exposure on the variability in metal bioaccumulation in *Mytilus edulis*. Environ Pollut. 144:272–279.

Munday PL, Warner RR, Monro K, Pandolfi JM, Marshall DJ. 2013. Predicting evolutionary responses to climate change in the sea. Ecol Lett. 16:1488–1500.

Munro AD, Pitcher TJ. 1985. Steroid hormones and agonistic behavior in a cichlid teleost, *Aequidens pulcher*. Horm Behav. 19:353–371.

Nadler LE, Killen SS, Mcclure EC, Munday PL, Mccormick MI. 2016. Shoaling reduces metabolic rate in a gregarious coral reef fish species. J Exp Biol. 219:2802–2805.

Nadler LE, Killen SS, Mccormick MI, Watson S, Munday PL. 2016. Effect of elevated carbon dioxide on shoal familiarity and metabolism in a coral reef fish. Conserv Physiol. 4:1–13.

Naguib M, van Oers K, Braakhuis A, Griffioen M, de Goede P, Waas JR. 2013. Noise annoys: effects of noise on breeding great tits depend on personality but not on noise characteristics. Anim Behav. 85:949–956.

Näslund J, Johnsson JI. 2016. State-dependent behavior and alternative behavioral strategies in brown trout (*Salmo trutta L.*) fry. Behav Ecol Sociobiol. 70:2111–2125.

Nedelec SL, Campbell J, Radford AN, Simpson SD, Merchant ND. 2016a. Particle motion: the missing link in underwater acoustic ecology. Methods Ecol Evol. 7:836–842.

Nedelec SL, Mills SC, Lecchini D, Nedelec B, Simpson SD, Radford AN. 2016b. Repeated exposure to noise increases tolerance in a coral reef fish. Environ Pollut. 216:428–436.

Nedelec SL, Radford AN, Pearl L, Nedelec B, Mccormick MI, Meekan MG, Simpson SD. 2017a. Motorboat noise impacts parental behaviour and offspring survival in a reef fish. Proc R Soc B. 284:20170134.

Nedelec SL, Mills SC, Radford AN, Beldade R, Simpson SD, Nedelec B, Côté IM. 2017b. Motorboat noise disrupts co-operative interspecific interactions. Sci Rep. 7:6987.

Nedelec SL, Radford AN, Simpson SD, Nedelec B, Lecchini D, Mills SC. 2014. Anthropogenic noise

playback impairs embryonic development and increases mortality in a marine invertebrate. Sci Rep. 4:5891.

Nedelec SL, Simpson SD, Morley EL, Nedelec B, Radford AN. 2015. Impacts of regular and random noise on the behaviour, growth and development of larval Atlantic cod (*Gadus morhua*). Proc R Soc B. 282:20151943.

Nedwell J, Turnpenny A, Langworthy J, Edwards B. 2003. Measurements of underwater noise during piling at the Red Funnel Terminal, Southampton, and observations of its effect on caged fish. Subacoustics LTD. Report 558 R 0207.

Nemeth E, Pieretti N, Zollinger SA, Geberzahn N, Partecke J, Mirand AC, Brumm H. 2013. Bird song and anthropogenic noise: vocal constraints may explain why birds sing higher-frequency songs in cities. Proc R Soc B. 280:20122798 .

Neo YY, Hubert J, Bolle L, Winter HV, ten Cate C, Slabbekoorn H. 2016. Sound exposure changes European seabass behaviour in a large outdoor floating pen: effects of temporal structure and a ramp-up procedure. Environ Pollut. 214:26–34.

Neo YY, Hubert J, Bolle LJ, Winter H V, Slabbekoorn H. 2018. European seabass respond more strongly to noise exposure at night and habituate over repeated trials of sound exposure. Environ Pollut. 239:367–374.

Neo YY, Seitz J, Kastelein RA, Winter HV, ten Cate C, Slabbekoorn H. 2014. Temporal structure of sound affects behavioural recovery from noise impact in European seabass. Biol Conserv. 178:65–73.

Neo YY, Ufkes E, Kastelein RA, Winter H V, ten Cate C, Slabbekoorn H. 2015. Impulsive sounds change European seabass swimming patterns: influence of pulse repetition interval. Mar Pollut Bull. 97:111–117.

Neumann B, Vafeidis AT, Zimmermann J, Nicholls RJ, Schmidt J, Doorn E van. 2015. Future coastal population growth and exposure to sea-level rise and coastal flooding - A global assessment. PLoS One. 10:e0118571.

Nishimura M, Shimizu E, Amakasu K, Du Y, Ode T, Takamasa T. 2013. Underwater noise property of water jet propelled electric boat. OCEANS 2013 MTS/IEEE Bergen. p. 1–5.

Nordt A, Klenke R. 2013. Sleepless in town - drivers of the temporal shift in dawn song in urban European blackbirds. PLoS One. 8:e71476.

Normandeau Associates. 2012. Effects of noise on fish, fisheries, and invertebrates in the U.S. Atlantic and Arctic from energy industry sound-generating activities. A workshop report for the US dept of the interior, Bureau of Ocean Energy Management, Herndon, VA.

Nowacek DP, Clark CW, Mann D, Miller PJ, Rosenbaum HC, Golden JS, Jasny M, Kraska J, Southall BL.

2015. Marine seismic surveys and ocean noise: time for coordinated and prudent planning. Front Ecol Environ. 13:378–386.

Ohlhorst SL, Liddell WD, Taylor RJ, Taylor JM. 1988. Evaluation of reef census techniques. Proc Sixth Int Coral Reef Symp. Townsville, Australia. 2:319–324.

Okumura T, Akamatsu T, Yan HY. 2002. Analyses of small tank acoustics: empirical and theoretical approaches. Bioacoustics. 12:330–332.

Oliva-Paterna FJ, Miñano PA, Torralva M. 2003. Habitat quality affects the condition of *Barbus sclateri* in Mediterranean semi-arid streams. Environ Biol Fishes. 67:13–22.

Oney B, Reineking B, O'Neill G, Kreyling J. 2013. Intraspecific variation buffers projected climate change impacts on *Pinus contorta*. Ecol Evol. 3:437–449.

Ortiz-Santaliestra ME, Marco A, Fernández MJ, Lizana M. 2006. Influence of developmental stage on sensitivity to ammonium nitrate of aquatic stages of amphibians. Environ Toxicol Chem. 25:105–111.

Ottersen G, Hjermann DØ, Stenseth NC. 2006. Changes in spawning stock structure strengthen the link between climate and recruitment in a heavily fished cod (*Gadus morhua*) stock. Fish Oceanogr. 15:230–243.

Owen MA, Swaisgood RR, Czekala NM, Steinman K, Lindburg DG. 2004. Monitoring stress in captive giant pandas (*Ailuropoda melanoleuca*): behavioral and hormonal responses to ambient noise. Zoo Biol. 23:147–164.

Oxman DS, Barnett-Johnson R, Smith ME, Coffin A, Miller DL, Josephson R, Popper AN. 2007. The effect of vaterite deposition on sound reception, otolith morphology, and inner ear sensory epithelia in hatchery-reared Chinook salmon (*Oncorhynchus tshawytscha*). Can J Fish Aquat Sci. 64:1469–1478.

Palanza P, Parmigiani S. 2017. How does sex matter? Behavior, stress and animal models of neurobehavioral disorders. Neurosci Biobehav Rev. 76:134–143.

Paris JR, King RA, Stevens JR. 2015. Human mining activity across the ages determines the genetic structure of modern brown trout (*Salmo trutta* L.) populations. Evol Appl. 8:573–585.

Parrott JL, Blunt BR. 2005. Life-cycle exposure of fathead minnows (*Pimephales promelas*) to an ethinylestradiol concentration below 1 ng/L reduces egg fertilization success and demasculinizes males. Environ Toxicol. 20:131–141.

Pecl GT, Araújo MB, Bell JD, Blanchard J, Bonebrake TC, Chen IC, Clark TD, Colwell RK, Danielsen F, Evengård B, et al. 2017. Biodiversity redistribution under climate change: impacts on ecosystems and human well-being. Science. 355:eaai9214.

Pespeni MH, Sanford E, Gaylord B, Hill TM, Hosfelt JD, Jaris HK, LaVigne M, Lenz EA, Russell AD,

Young MK, et al. 2013. Evolutionary change during experimental ocean acidification. Proc Natl Acad Sci USA. 110:6937–6942.

Pine MK, Schmitt P, Culloch RM, Lieber L, Kregting LT. 2019. Providing ecological context to anthropogenic subsea noise: assessing listening space reductions of marine mammals from tidal energy devices. Renew Sustain Energy Rev. 103:49–57.

Planque B, Fromentin J-M, Cury P, Drinkwater KF, Jennings S, Perry RI, Kifani S. 2010. How does fishing alter marine populations and ecosystems sensitivity to climate? J Mar Syst. 79:403–417.

Popper A, Fay R. 1993. Sound detection and processing by fish: critical review and major research questions. Brain Behav Evol. 41:14–38.

Popper AN. 2003. Effects of anthropogenic sounds on fishes. Fisheries. 28:24–31.

Popper AN, Fay RR. 2011. Rethinking sound detection by fishes. Hear Res. 273:25–36.

Popper AN, Hawkins AD. 2019. An overview of fish bioacoustics and the impacts of anthropogenic sounds on fishes. J Fish Biol. 94:692–713.

Pörtner HO, Knust R. 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science. 315:95–97.

Post DM, Palkovacs EP, Schielke EG, Dodson SI. 2008. Intraspecific variation in a predator affects community structure and cascading trophic interactions. Ecology. 89:2019–2032.

Pottinger TG, Carrick TR, Hughes SE, Balm PHM. 1996. Testosterone, 11-ketotestosterone, and estradiol-17β modify baseline and stress-induced interrenal and corticotropic activity in trout. Gen Comp Endocrinol. 104:284–295.

Potvin DA, Macdougall-Shackleton SA. 2015. Traffic noise affects embryo mortality and nestling growth rates in captive zebra finches. J Exp Zool. 323:722–730.

Poulton DA, Porteus CS, Simpson SD. 2016. Combined impacts of elevated CO_2 and anthropogenic noise on European sea bass (*Dicentrarchus labrax*). ICES J Mar Sci. 74:1230–1236.

Powell DM, Carlstead K, Tarou LR, Brown JL, Monfort SL. 2006. Effects of construction noise on behavior and cortisol levels in a pair of captive giant pandas (*Ailuropoda melanoleuca*). Zoo Biol. 25:391–408.

Pratchett MS, Munday PL, Wilson SK, Graham NAJ, Cinneri JE, Bellwood DR, Jones GP, Polunin NVC, McClanahan TR. 2008. Effects of climate-induced coral bleaching on coral-reef fishes - Ecological and economic consequences. Oceanogr Mar Biol An Annu Rev. 46:251–296.

Pratchett MS, Wilson SK, Berumen ML, McCormick MI. 2004. Sublethal effects of coral bleaching on an obligate coral feeding butterflyfish. Coral Reefs. 23:352–356.

Pruitt JN, Cote J, Ferrari MCO. 2012. Behavioural trait variants in a habitat-forming species dictate the nature of its interactions with and among heterospecifics. Funct Ecol. 26:29–36.

Purser J, Bruintjes R, Simpson SD, Radford AN. 2016. Condition-dependent physiological and behavioural responses to anthropogenic noise. Physiol Behav. 155:157–161.

Purser J, Radford AN. 2011. Acoustic noise induces attention shifts and reduces foraging performance in three-spined sticklebacks (*Gasterosteus aculeatus*). PLoS One. 6:e17478.

Pye S, Li FGN, Price J, Fais B. 2017. Achieving net-zero emissions through the reframing of UK national targets in the post-Paris agreement era. Nat Energy. 2:17024.

Quadros S, Goulart VDL, Passos L, Vecci MAM, Young RJ. 2014. Zoo visitor effect on mammal behaviour: does noise matter? Appl Anim Behav Sci. 156:78–84.

Raap T, Pinxten R, Casasole G, Dehnhard N, Eens M. 2017. Ambient anthropogenic noise but not light is associated with the ecophysiology of free-living songbird nestlings. Sci Rep. 7:2754.

Radford AN, Kerridge E, Simpson SD. 2014. Acoustic communication in a noisy world: can fish compete with anthropogenic noise? Behav Ecol. 25:1022–1030.

Radford AN, Purser J, Bruintjes R, Voellmy IK, Everley KA, Wale MA, Holles S, Simpson SD. 2016a. Beyond a simple effect: variable and changing responses to anthropogenic noise. In: Popper AN, Hawkins A, editors. Effects of Noise on Aquatic Life II. Advances in Experimental Medicine and Biology. New York, NY: Springer. p. 901–907.

Radford AN, Lèbre L, Lecaillon G, Nedelec SL, Simpson SD. 2016b. Repeated exposure reduces the response to impulsive noise in European seabass. Glob Chang Biol. 22:3349–3360.

Radford C, Montgomery J, Caiger P. 2012. Pressure and particle motion detection thresholds in fish: a re-examination of salient auditory cues in teleosts. J Exp Biol. 215:3429–3435.

Raffard A, Santoul F, Cucherousset J, Blanchet S. 2019. The community and ecosystem consequences of intraspecific diversity: a meta-analysis. Biol Rev. 94:648–661.

Randall JE. 1961. A contribution to the biology of the convict surgeonfish of the Hawaiian Islands, *Acanthurus triostegus sandoicensis*. Pacific Sci. 15:215–272.

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ. 2007. Integrating animal temperament within ecology and evolution. Biol Rev. 82:291–318.

Reid ML, Purcell JRC. 2011. Condition-dependent tolerance of monoterpenes in an insect herbivore. Arthropod Plant Interact. 5:331–337.

Reimer T, Dempster T, Warren-Myers F, Jensen AJ, Swearer SE. 2016. High prevalence of vaterite in sagittal otoliths causes hearing impairment in farmed fish. Sci Rep. 6:25249.

Relyea RA, Mills N. 2001. Predator-induced stress makes the pesticide carbaryl more deadly to gray treefrog tadpoles (*Hyla versicolor*). Proc Natl Acad Sci USA. 98:2491–2496.

Roberts L, Pérez-Domínguez R, Elliott M. 2016. Use of baited remote underwater video (BRUV) and motion analysis for studying the impacts of underwater noise upon free ranging fish and

implications for marine energy management. Mar Pollut Bull. 112:75–85.

Rodgers G, Tenzing P, Clark T. 2016. Experimental methods in aquatic respirometry: the importance of mixing devices and accounting for background respiration. J Fish Biol. 88:65–80.

Ros AFH, Vullioud P, Bruintjes R, Vallat A, Bshary R. 2014. Intra- and interspecific challenges modulate cortisol but not androgen levels in a year-round territorial damselfish. J Exp Biol. 217:1768–1774.

Rossington K, Benson T, Lepper P, Jones D. 2013. Eco-hydro-acoustic modeling and its use as an EIA tool. Mar Pollut Bull. 75:235–243.

Rouzé H, Lecellier G, Langlade MJ, Planes S, Berteaux-Lecellier V. 2015. Fringing reefs exposed to different levels of eutrophication and sedimentation can support similar benthic communities. Mar Pollut Bull. 92:212–221.

Royauté R, Pruitt JN. 2015. Varying predator personalities generates contrasting prey communities in an agroecosystem. Ecology. 96:2902–2911.

Rudman SM, Rodriguez-Cabal MA, Stier A, Sato T, Heavyside J, El-Sabaawi RW, Crutsinger GM. 2015. Adaptive genetic variation mediates bottom-up and top-down control in an aquatic ecosystem. Proc R Soc B. 282:20151234.

Russell R, Guerry AD, Balvanera P, Gould RK, Basurto X, Chan KMA, Klain S, Levine J, Tam J. 2013. Humans and nature: how knowing and experiencing nature affect well-being. Annu Rev Environ Resour. 38:473–502.

Sabet SS, Neo YY, Slabbekoorn H. 2015. The effect of temporal variation in sound exposure on swimming and foraging behaviour of captive zebrafish. Anim Behav. 107:49–60.

Sabet SS, Wesdorp K, van Dooren D, Slabbekoorn H. 2016a. Sound affects behavior of captive zebrafish: always consider the potential for acoustic effects on your laboratory tests. Proc Mtgs Acoust. 27:010010.

Sabet SS, Van Dooren D, Slabbekoorn H. 2016b. Son et lumiere: sound and light effects on spatial distribution and swimming behavior in captive zebrafish. Environ Pollut. 212:480–488.

Sambrook K, Jones G, Bonin M. 2016. Life on the edge: Coral reef fishes exhibit strong responses to a habitat boundary. Mar Ecol Prog Ser. 561:203–215.

Sardain A, Sardain E, Leung B. 2019. Global forecasts of shipping traffic and biological invasions to 2050. Nat Sustain. 2:274–282.

Scholik AR, Yan HY. 2002. Effects of boat engine noise on the auditory sensitivity of the fathead minnow, *Pimephales promelas*. Environ Biol Fishes. 63:203–209.

Schrandt MN, Hardy KM, Johnson KM, Lema SC. 2011. Physical habitat and social conditions across a

coral reef shape spatial patterns of intraspecific behavioral variation in a demersal fish. Mar Ecol. 33:149–164.

Schrandt MN, Lema SC. 2011. Habitat-associated intraspecific variation in behavior and stress responses in a demersal coral reef fish. Mar Ecol Prog Ser. 443:153–166.

Schreck CB, Tort L. 2016. The concept of stress in fish. In Schreck CB, Tort L, Farrell AP, Brauner CJ, editors. Biology of Stress in Fish: Fish Physiology. Vol. 35. Cambridge, USA: Academic Press. p. 1–34.

Schwarz A. 1974. The inhibition of aggressive behavior by sound in the cichlid fish, *Cichlasoma centrarchus*. Z Tierpsychol. 35:508–517.

Sebastianutto L, Picciulin M, Costantini M, Ferrero EA. 2011. How boat noise affects an ecologically crucial behaviour: the case of territoriality in *Gobius cruentatus* (Gobiidae). Environ Biol Fish. 92:207–215.

Sebens KP. 1987. The ecology of indeterminate growth in animals. Annu Rev Ecol Syst. 18:371–407.

Senzaki M, Kadoya T, Francis CD, Ishiyama N, Nakamura F. 2018. Suffering in receivers: negative effects of noise persist regardless of experience in female anurans. Funct Ecol. 32:2054–2064.

Sertlek HÖ, Slabbekoorn H, ten Cate C, Ainslie MA. 2019. Source specific sound mapping: spatial, temporal and spectral distribution of sound in the Dutch North Sea. Environ Pollut. 247:1143–1157.

Shannon G, Mckenna MF, Angeloni LM, Crooks KR, Fristrup KM, Brown E, Warner KA, Nelson MD, White C, Briggs J, et al. 2015. A synthesis of two decades of research documenting the effects of noise on wildlife. Biol Rev. 91:982–1005.

Shepherdson DJ, Carlstead KC, Wielebnowski N. 2004. Cross-insitutional assessment of stress responses in zoo animals using longitudinal monitoring of faecal corticoids and behaviour. Anim Welf. 13:S105–S113.

Shima JS, Osenberg CW. 2003. Cryptic density dependence: effects of covariation between density and site quality in reef fish. Ecology. 84:46–52.

Shine R. 1988. The evolution of large body size in females: a critique of Darwin's "fecundity advantage" model. Am Nat. 131:124–131.

Siefert A, Ritchie ME. 2016. Intraspecific trait variation drives functional responses of old-field plant communities to nutrient enrichment. Oecologia. 181:245–255.

Sih A, Bell AM, Johnson JC, Ziemba RE. 2004. Behavioral syndromes: an integrative overview. Q Rev Biol. 79:241–277.

Sih A, Cote J, Evans M, Fogarty S, Pruitt J. 2012. Ecological implications of behavioural syndromes. Ecol Lett. 15:278–289.

Sih A, Del Giudice M. 2012. Linking behavioural syndromes and cognition: A behavioural ecology perspective. Phil Trans R Soc B. 367:2762–2772.

Sih A. 2013. Understanding variation in behavioural responses to human-induced rapid environmental change: a conceptual overview. Anim Behav. 85:1077–1088.

Simpson SD, Meekan M, Montgomery J, McCauley R, Jeffs A. 2005. Homeward sound. Science. 308:221.

Simpson SD, Purser J, Radford AN. 2015. Anthropogenic noise compromises antipredator behaviour in European eels. Glob Chang Biol. 21:586–593.

Simpson SD, Radford AN, Nedelec SL, Ferrari MCO, Chivers DP, McCormick MI, Meekan MG. 2016a. Anthropogenic noise increases fish mortality by predation. Nat Commun. 7:10544.

Simpson SD, Radford AN, Holles S, Ferarri MCO, Chivers DP, McCormick MI, Meekan MG. 2016b. Small-boat noise impacts natural settlement behavior of coral reef fish larvae. In: Popper AN, Hawkins AD, editors. Effects of Noise on Aquatic Life II. Advances in Experimental Medicine and Biology. New York, NY: Springer. p. 1041–1048.

Sinsch U. 1992. Sex-biased site fidelity and orientation behaviour in reproductive natterjack toads (*Bufo calamita*). Ethol Ecol Evol. 4:15–32.

Skagen SK, Melcher CP, Muths E. 2006. The interplay of habitat change, human disturbance and species interactions in a waterbird colony. Am Midl Nat. 145:18–28.

Skinner MK. 2015. Environmental epigenetics and a unified theory of the molecular aspects of evolution: a neo-Lamarckian concept that facilitates neo-Darwinian evolution. Genome Biol Evol. 7:1296–1302.

Slabbekoorn H, Bouton N, van Opzeeland I, Coers A, ten Cate C, Popper AN. 2010. A noisy spring: the impact of globally rising underwater sound levels on fish. Trends Ecol Evol. 25:419–427.

Slabbekoorn H. 2016. Aiming for progress in understanding underwater noise impact on fish: complementary need for indoor and outdoor studies. In: Popper AN, Hawkins AD, editors. Effects of Noise on Aquatic Life II. Advances in Experimental Medicine and Biology. New York, NY: Springer. p. 1057–1065.

Smith M, Kane A, Popper AN. 2004. Noise-induced stress response and hearing loss in goldfish (*Carassius auratus*). J Exp Biol. 207:427–435.

Sokolova IM, Frederich M, Bagwe R, Lannig G, Sukhotin AA. 2012. Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. Mar Environ Res. 79:1–15.

Sokolova IM. 2013. Energy-limited tolerance to stress as a conceptual framework to integrate the effects of multiple stressors. Integr Comp Biol. 53:597–608.

de Soto NA, Delorme N, Atkins J, Howard S, Williams J, Johnson M. 2013. Anthropogenic noise causes body malformations and delays development in marine larvae. Sci Rep. 3:2831.

Spalding MD, Ravilious C, Green EP. 2001. World atlas of coral reefs. Berkeley, USA: University California Press.

Spear LB, Ainley DG, Ribic CA. 1996. Incidence of plastic in seabirds from the tropical Pacific, 1984-91: relation with distribution of species, sex, age, season, year and body weight. Mar Environ Res. 40:123–146.

Spiga I, Aldred N, Caldwell GS. 2017. Anthropogenic noise compromises the anti-predator behaviour of the European seabass, *Dicentrarchus labrax (L.)*. Mar Pollut Bull. 122:297–305.

Stearns SC. 1992. The Evolution of Life Histories. Oxford: Oxford University Press.

Streelman J, Gmyrek SL, Kidd MR, Kidd C, Robinson RL, Hert E, Ambali AJ, Kocher TD. 2004. Hybridization and contemporary evolution in an introduced cichlid fish from Lake Malawi National Park. Mol Ecol. 13:2471–2479.

Sweeting RM, Beamish RJ, Neville CM. 2004. Crystalline otoliths in teleosts: comparisons between hatchery and wild coho salmon (*Oncorhynchus kisutch*) in the Strait of Georgia. Rev Fish Biol Fish. 14:361–369.

The European Parliament and the Council of the European Union. 2008. Directive 2008/56/EC of the European Parliament and of the Council.

Thompson PM, Brookes KL, Graham IM, Barton TR, Needham K, Bradbury G, Merchant ND. 2013. Short-term disturbance by a commercial two-dimensional seismic survey does not lead to long-term displacement of harbour porpoises. Proc R Soc B. 280:20132001.

Thorson JT, Munch SB, Cope JM, Gao J. 2017. Predicting life history parameters for all fishes worldwide. Ecol Appl. 27:2262–2276.

Tixier P, Gasco N, Duhamel G, Guinet C. 2015. Habituation to an acoustic harassment device (AHD) by killer whales depredating demersal longlines. ICES J Mar Sci. 72:1673–1681.

Tricas TC, Boyle KS. 2014. Acoustic behaviors in Hawaiian coral reef fish communities. Mar Ecol Prog Ser. 511:1–16.

Turner GF, Seehausen O, Knight ME, Allender CJ, Robinson RL. 2001. How many species of cichlid fishes are there in African lakes? Mol Ecol. 10:793–806.

Valladares F, Matesanz S, Guilhaumon F, Araújo MB, Balaguer L, Benito-Garzón M, Cornwell W, Gianoli E, van Kleunen M, Naya DE, et al. 2014. The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. Ecol Lett. 17:1351–1364.

Veirs S, Veirs V, Wood JD. 2016. Ship noise extends to frequencies used for echolocation by endangered killer whales. PeerJ. 4:e1657.

Venturini S, Massa F, Castellano M, Fanciulli G, Povero P. 2018. Recreational boating in the Portofino Marine Protected Area (MPA), Italy: characterization and analysis in the last decade (2006–2016) and some considerations on management. Mar Policy. In Press.

Voellmy IK, Purser J, Flynn D, Kennedy P, Simpson SD, Radford AN. 2014a. Acoustic noise reduces foraging success in two sympatric fish species via different mechanisms. Anim Behav. 89:191–198.

Voellmy IK, Purser J, Simpson SD, Radford AN. 2014b. Increased noise levels have different impacts on the anti-predator behaviour of two sympatric fish species. PLoS One. 9:e102946.

Wale MA, Simpson SD, Radford AN. 2013a. Size-dependent physiological responses of shore crabs to single and repeated playback of ship noise. Biol Lett. 9:20121194.

Walther G-R, Post E, Convey P, Menzel A, Parmesan C, Beebee TJC, Fromentin J-M, Hoegh-Guldberg O, Bairlein F. 2002. Ecological responses to recent climate change. Nature. 416:389–395.

Washburn DA, Taglialatela LA. 2012. Attention as it is manifest across species. In: Wasserman EA, editor. Comparative Cognition: Experimental Explorations of Animal Intelligence. Oxford, UK: Oxford University Press. p. 127–142.

Wecke C, Liebert F. 2013. Improving the reliability of optimal in-feed amino acid ratios based on individual amino acid efficiency data from N balance studies in growing chicken. Animals. 3:558–573.

Weimann SR, Black AN, Leese J, Richter ML, Itzkowitz M, Burger RM. 2018. Territorial vocalization in sympatric damselfish: acoustic characteristics and intruder discrimination. Bioacoustics. 27:87–102.

White JW, Cole BJ, Cherr GN, Connon RE, Brander SM. 2017. Scaling up endocrine disruption effects from individuals to populations: outcomes depend on how many males a population needs. Environ Sci Technol. 51:1802–1810.

Whitfield AK, Becker A. 2014. Impacts of recreational motorboats on fishes: a review. Mar Pollut Bull. 83:24–31.

Williams NM, Crone EE, Roulston TH, Minckley RL, Packer L, Potts SG. 2010. Ecological and life-history traits predict bee species responses to environmental disturbances. Biol Conserv. 143:2280–2291.

Williams R, Veirs S, Veirs V, Ashe E, Mastick N. 2019. Approaches to reduce noise from ships operating in important killer whale habitats. Mar Pollut Bull. 139:459–469.

Wilson S, Bellwood DR. 1997. Cryptic dietary components of territorial damselfishes

(Pomacentridae, Labroidei). Mar Ecol Prog Ser. 153:299–310.

Wilson SK, Graham NAJ, Pratchett MS, Jones GP, Polunin NVC. 2006. Multiple disturbances and the global degradation of coral reefs: Are reef fishes at risk or resilient? Glob Chang Biol. 12:2220–2234.

Winberg S, Nilsson GE. 1993. Roles of brain monoamine neurotransmitters in agonistic behaviour and stress reactions, with particular reference to fish. Comp Biochem Physiol Part C Comp. 106:597–614.

Wong BBM, Candolin U. 2015. Behavioral responses to changing environments. Behav Ecol. 26:665–673.

Wright KJ, Higgs DM, Leis JM. 2011. Ontogenetic and interspecific variation in hearing ability in marine fish larvae. Mar Ecol Prog Ser. 424:1–13.

Wysocki LE, Davidson J, Smith M, Frankel A. 2007. Effects of aquaculture production noise on hearing, growth, and disease resistance of rainbow trout *Oncorhynchus mykiss*. Aquaculture. 27:687–697.

Wysocki LE, Dittami J, Ladich F. 2006. Ship noise and cortisol secretion in European freshwater fishes. Biol Conserv. 128:501–508.

Wysocki LE, Ladich F. 2005. Hearing in fishes under noise conditions. J Assoc Res Otolaryngol. 6:28–36.

Zera AJ, Harshman LG. 2001. The physiology of life history trade-offs in animals. Annu Rev Ecol Syst. 32:95–126.

Appendix

Appendix A: Additional material for Chapter 1

Methodology – Systematic literature review

We conducted a systematic review of the peer-reviewed literature; see Figure A.1 for a schematic of our methodology. Initially, we used "ANTHROPOGENIC NOISE" OR "ACOUSTIC DISTURBANC*" OR "NOISE POLLUT*" OR "MAN-MADE NOIS*" as search terms in Thompson's *ISI Web of Science*, to find potentially relevant papers published between 1900 and 2018 (n = 3,309 records). In addition, we conducted a search in *Web of Science* using the same methodology as Shannon *et al*. (2015)—the most comprehensive recent review on the impacts of anthropogenic noise on non-human animals—but for 2013–2018 (the years since their search), which produced 1,516 records. Alongside these database searches, we included the 242 papers cited in the Shannon *et al*. (2015) review and papers that have cited Shannon *et al*. (2015) (n = 69; as of 05/10/2018 in *Web of Science*) in our initial list. Subsequent selection steps followed the Prisma protocol for systematic reviews (Moher et al. 2009). We used JabRef (http://www.jabref.org/) bibliography reference manager to check the combined records (n = 5,136) for duplicates, resulting in 4,558 unique records for the screening phase. We then screened papers for those that focussed on the impact of anthropogenic noise on non-human animals, based on a review of the title and abstract. Our criteria for noise selection were that studies had used real anthropogenic noise sources, or their playback equivalents, or synthetic noise; we excluded studies using pure tones as an acoustic disturbance as these are generally used in hearing assessments or as stimuli for training. We also excluded reviews, modelling studies (predicting risk factors from known hearing thresholds), experimental studies with a sample size of 1, and observational studies where impacts of noise were suggested but noise measurements not taken. Overall, the screening phase retained 589 records, which we then fully assessed to identify observational and experimental studies testing intraspecific variation in non-human animal responses to anthropogenic noise.

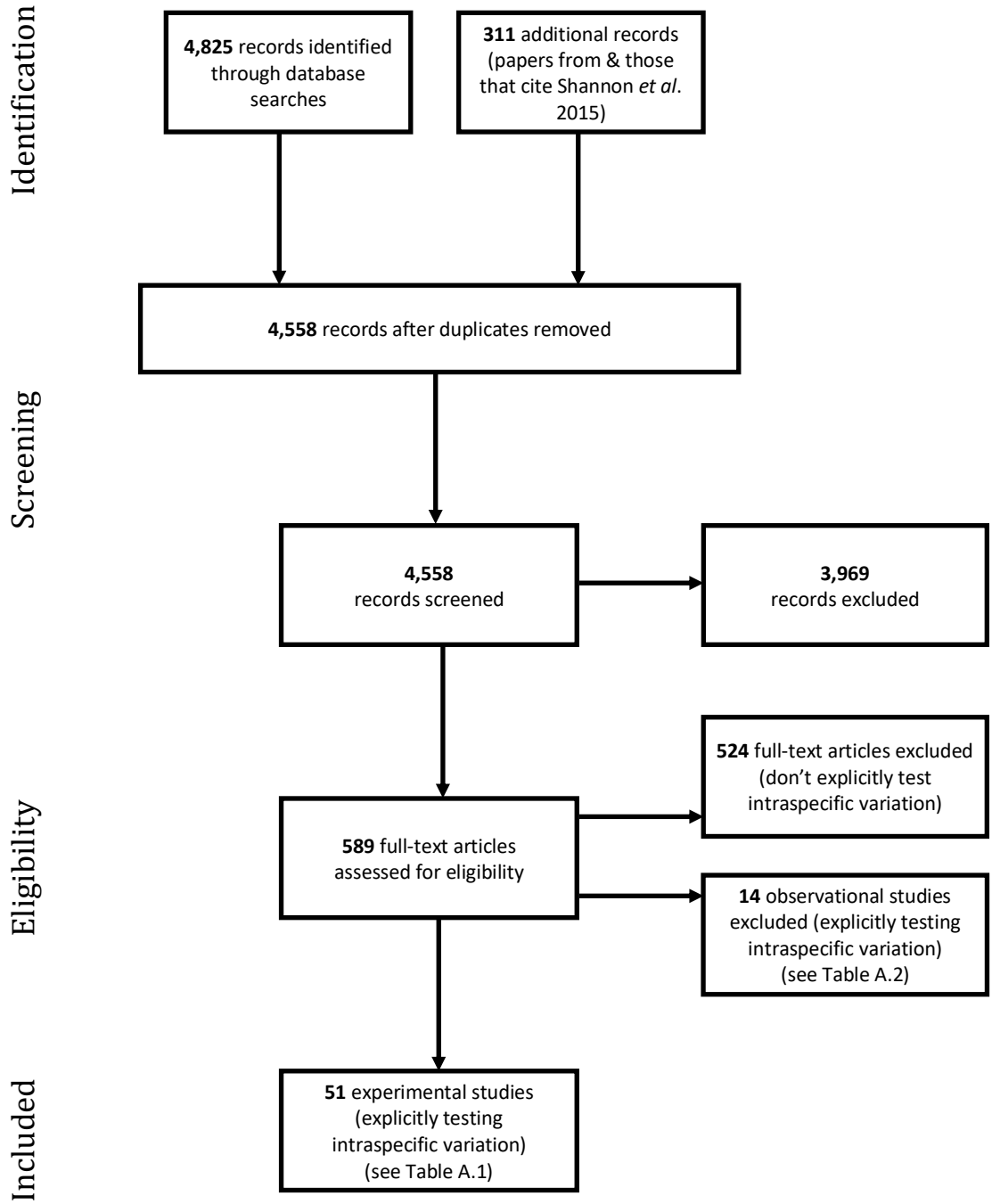

Figure A.1. PRIMSA flow diagram outlining the systematic literature review process.

Methodology – Effect-size calculations

Where possible for studies in Table A.1, we calculated standardised effect sizes (*Hedge's g*) for each treatment group, correcting for small sample size bias, using the compute.es package in R (R Core Team, 2016; www.R-project.org). Effect sizes were calculated for all studies where the means, sample sizes and standard error or standard deviation were presented or were accessible from the raw data, or where independent statistical tests per condition were available. Effect sizes for studies using a within-subjects design were only calculated if the correlation between the repeated measures were accessible (Borenstein 2009). In addition, WebPlotDigitizer (https://automeris.io/WebPlotDigitizer/) was used to extract these metrics from paper figures when they were not included in the text, with such software shown to provide high accuracy and validity when extracting data from plots (Drevon et al. 2017). For instances where multiple response measures considering intraspecific variation were shown to be significant or where there was no effect found in any of those response measures, a single example was used (six out of 15; 40%). Further, where possible, we determined an overall (composite) effect size of each intrinsic characteristic and extrinsic factor for each paper, to enable quantitative assessment of the relative potential importance of each source of intraspecific variation. Composite effect sizes were determined following the methods of Borenstein *et al.* (2009) on computing combined effects within individual studies (see Figure 1.3).

Table A.1. Experimental studies investigating intraspecific variation in responses to anthropogenic noise as a consequence of (a) intrinsic characteristics and (b) extrinsic factors, as reported from the primary research papers. Examples use either real or playback of anthropogenic noise, or playback of either pink, white or brown noise as an acoustic disturbance. Effect size statistics are in bold and represent the standardised mean difference, corrected for small sample size bias, and the corresponding confidence intervals as calculated from available information. The direction for each individual effect size are presented so that positive integers represent anthropogenic noise increasing the response measure, and negative integers causing a decrease in response.

Intraspecific variation	Examples	References
(a) Intrinsic characteristics		
Body size/ Age	Hybrid striped bass (white bass (*Morone chrysops*) x striped bass (*Morone saxatilis*)) of larger size suffered more severe internal injuries than smaller individuals when exposed to pile-driving noise playback.	(Casper et al. 2013)
	Shore crabs (*Carcinus maenas*) of larger size had disproportionately higher oxygen-consumption rates than smaller conspecifics in response to ship-noise playback compared to ambient-sound controls.	(Wale et al. 2013)
	Younger birds of various species showed greater avoidance than older individuals when exposed to traffic-noise playback, with the capture ratio of young/adult birds declining in locations exposed to traffic noise.	(McClure et al. 2017)
	Zebra finch (*Taenopygia guttata*) post-fledglings had a greater reduction in telomere length (**Hedge's *g*: -0.68; CI: -1 – -0.35**) than pre-fledglings (**Hedge's *g*: -0.04; CI: -0.32–0.23**) when exposed to traffic-noise playback.	(Dorado-Correa et al. 2018)
	*Seabass (*Dicentrarchus labrax) of smaller size startled in response to playback of pile-driving noise at a lower sound level than larger conspecifics.	(Kastelein et al. 2017)
	California sea lions (*Zalophus californianus*) exposed to mid-frequency sonar exhibited a dose-response relationship with increasing sound levels; however, the probability of response at lower exposure levels declined with the removal of individuals under the age of two years.	(Houser et al. 2013a)
Body condition	European eels (*Anguilla anguilla*) in poorer condition exhibited a reduced response to a simulated predatory strike when played ship noise compared to ambient sound **(Hedge's *g*: -0.64; CI: -1.28– -0.01)**, whereas better-quality individuals showed no such noise effect **(Hedge's *g*: -0.18; CI: -0.79–0.42)**.	(Purser et al. 2016)
	Zebra finch (*Taeniopygia guttata*) offspring survival rates when exposed to chronic traffic noise tended to increase when the maternal baseline corticosterone levels were higher, whereas no similar trend was found in survival rate under no noise conditions.	(Potvin and Macdougall-Shackleton 2015)
Sex	Daffodil cichlid (*Neolamprologus pulcher*) dominant males removed sand from their nests fewer times during boat-noise playback than in ambient-sound conditions, whereas dominant females showed no sound-treatment difference in sand-digging frequency.	(Bruintjes and Radford 2013)
	Female wild mice (*Mus musculus*) did not differ in their corticosterone levels when exposed to low-frequency mining noise compared to control conditions, whereas males had higher corticosterone levels in mining-noise conditions compared to control conditions	(Mancera et al. 2017)

	Spiny lobster (*Jasus edwardsii*) females and males both had lower total haemocyte counts after being exposed to sound from a seismic air gun compared to ambient controls.	(Fitzgibbon et al. 2017)
	Tree swallow (*Tachycineta bicolor*) female settlement was delayed by an increase in background traffic noise, whereas there was no noise-induced delay in male settlement.	(Injaian et al. 2018)
	Greater sage-grouse (*Centrocercus urophasianus*) male abundance declined on leks exposed to drilling or traffic noise compared to ambient control leks, with weaker, but similar, effects of noise playback on female abundance.	(Blickley et al. 2012)
	Rat (*Rattus sp.*) males exposed to vibrational noise spent less time sitting post-noise exposure compared to control rats, whereas females spent more time sitting post-noise exposure compared to the control group.	(Avaliani et al. 2018)
	Great tit (*Parus major)* males with low exploratory scores avoided the white-noise playback more than males with high exploratory scores, this effect was reversed in females.	(Naguib et al. 2013)
	Mediterranean spiny lobster (*Palinurus elephas*) males and females both had elevated expression levels of the Hsp70 protein (% integrated density value) when exposed to boat-noise playback compared to control conditions.	(Filiciotto et al. 2014)
Personality	Great tits (*Parus major*) characterised with high exploratory scores had a lower latency time to first nestbox visit than birds with low exploratory scores during white-noise playback.	(Naguib et al. 2013)

(b) Extrinsic factors

Context	Daffodil cichlids (*Neolamprologus pulcher*) showed no difference in anti-predator defence behaviours when exposed to boat-noise and ambient-sound playback when they had eggs in their nest **(Hedge's *g*: -0.29; CI: -1.15–0.57)**, but there was less defensive behaviour during boat-noise playback than ambient-sound playback in the absence of eggs **(Hedge's *g*: -0.79; CI: -1.54– -0.03)**.	(Bruintjes and Radford 2013)
	European sea bass (*Dicentrarchus labrax*) exposed to playback of impulsive noise (filtered brown noise) showed a greater increase in group cohesion at night than during the day.	(Neo et al. 2018)
	Largemouth bass (*Micropterus salmoides*) adults showed no significant difference in the number of turns over their nest (vigilance behaviour) during exposure to motorboat noise compared to the pre-treatment period, a consistent response was shown across three offspring developmental stages.	(Maxwell et al. 2018)
	Australian snapper (*Pagrus auratus*) inhabiting an open (unprotected) habitat decreased feeding activity and displayed avoidance behaviours during motorboat passes compared to pre- and post-sound periods, but these effects were absent in fish inhabiting a protected area.	(Mensinger et al. 2018)
	Gobies (*Gobius cruentatus)* were more submissive towards an intruder during boat-noise playback compared to a silent control when resident on a territory, whereas individuals acting as an intruder during a similar territorial encounter spent less time displaying submissive behaviours in the noise treatment compared to the silent control.	(Sebastianutto et al. 2011)

	House wren (*Troglodytes aedon*) males that were part of a pair displayed a higher peak frequency in their songs than unpaired males during 'pink' noise exposure.	(Grabarczyk et al. 2018)
	Atlantic herring (*Clupea harengus*) shoals of low density showed stronger avoidance behaviours than high-density shoals in response to vessel-noise playback.	(Handegard et al. 2015)
	House wren (*Troglodytes aedon)* adults inhabiting a rural environment had an increase in corticosterone following exposure to traffic-noise playback **(Hedge's g: 2.16; CI: 0.75–3.57)**, whereas urban conspecifics exhibited no such response to the noise exposure **(Hedge's g: 0.85; CI: -0.09–1.79)**.	(Davies et al. 2017)
	Tree frogs (*Hyla arborea*) singing alone did not adjust their singing bout duration during exposure to traffic-noise playback **(Hedge's *g*: -0.46; CI: -1.08–0.16)**, whereas a noise-induced change in calling activity was observed for tree frogs singing in a chorus **(Hedge's *g*: -1.09; CI: -1.9– -0.28)**.	(Lengagne 2008)
	Perch (*Perca fluviatilis*) in single-species enclosures reduced feeding attempts during exposure to motorboat noise, whereas no effect of motorboat-noise exposure was evident for perch in mixed-species enclosures with roach (*Rutilus rutilus*).	(Magnhagen et al. 2017)
	Blue whales (*Balaenotera musculus*) that were deep-feeding were affected by exposure to mid-frequency sonar, whereas whales in other behavioural states showed less response.	(Goldbogen et al. 2013)
	Mediterranean spiny lobsters (*Palinurus elephas*) in groups of four increased both their distance moved and velocity when exposed to boat-noise playback compared to control groups; no such increases were apparent when tested alone.	(Filiciotto et al. 2014)
	White-crowned sparrow (*Zonotrichia leucophrys*) males in urban areas decreased the bandwidth of their song when exposed to playback of experimental noise compared to before noise playback, whereas rural males did not adjust their song bandwidth.	(Gentry et al. 2017)
Repeated exposure	Ambon damselfish (*Pomacentrus amboinensis*) exposed to real motorboat noise showed immediate reductions in boldness relative to ambient-sound controls **(Hedge's *g*: -1.36; CI: -2.07– -0.66)**, but fish returned to pre-boat exposure behaviours within the 20-min trial **(Hedge's *g*: -0.02; CI:-0.65–0.62)**.	(Holmes et al. 2017)
	European sea bass (*Dicentrarchus labrax*) exposed to impulsive noise (filtered brown noise) swam faster, deeper and in tighter shoals compared to pre-exposure baseline levels, but recovered within the 60-min exposure period.	(Neo et al. 2015)
	Lined seahorses (*Hippocampus erectus*) exposed to high ambient-sound levels from aquarium machinery initially made more tail adjustments whilst stationary than those in quiet control tanks (**Hedge's *g*: 0.79; CI: -0.32–1.9)**, but this response difference diminished after three weeks **(Hedge's *g*: 0.05; CI: -0.96–1.06)**.	(Anderson et al. 2011)
	Threespot dascyllus (*Dascyllus trimaculatus*) exposed to motorboat-noise playback initially exhibited elevated ventilation rates compared to ambient controls **(Hedge's *g*: 1.68; CI: 0.96–2.39)**, but these responses lessened after one week of repeated exposure **(Hedge's *g*: 0.51; CI: -0.09–1.11)**.	(Nedelec et al. 2016b)
	Greater mouse-eared bats (*Myotis myotis*) displayed a higher proportion of responses when first exposed to short-term traffic-noise playback compared to a silent control, but the proportion of responses was reduced when bats were exposed to a second exposure.	(Luo et al. 2014)
	California sea lions (*Zalophus californianus*) exposed to mid-frequency sonar exhibited a dose-response relationship with increasing sound levels, and responses did not change over repeated exposures of the noise stimulus.	(Houser et al. 2013a)

	Bottlenose dolphins (*Tursiops truncatus*) exposed to mid-frequency sonar exhibited a dose-response relationship with increasing sound levels, however the probability of response at sound levels below 160 dB re 1 μPa decreased with repeated exposure of the noise stimulus.	(Houser et al. 2013b)
	American black ducks (*Anus rubripes*) reacted (alert & fleeing responses) to simulated aircraft overflight noise on first exposure, but the proportion of occasions that the birds reacted declined with repeated exposure to the noise stimulus.	(Conomy et al. 1998)
	Killer whales (*Orcinus orca*) increased their distance from the vessel when first exposed to an acoustic harassment device that was turned on rather than turned off, but the distance from the vessel no longer changed when the device was on vs off during successive exposures.	(Tixier et al. 2015)
	Atlantic bottlenose dolphins (*Tursiops truncatus*) showed reduced target-detection performance when first exposed to vibratory pile-driving noise playback compared to control periods, but no change in target-detection performance was evident by the second replication.	(Branstetter et al. 2018)
	Perch (*Perca fluviatilis*) in single-species enclosures made fewer feeding attempts during noise exposure compared to control trials, but over multiple days the number of feeding attempts increased in both treatments.	(Magnhagen et al. 2017)
	European sea bass (*Dicentrarchus labrax*) exposed to playback of filtered brown noise displayed increases in swimming depth, but these changes declined with repeated exposure over eight trials.	(Neo et al. 2018)
	Shore crabs (*Carcinus maenas*) exposed to ship-noise playback had a significantly higher oxygen-consumption rate compared to ambient controls, but responses did not change during repeated exposure over 16 days, whereas oxygen-consumption increased over the duration of multiple trials for the ambient control crabs.	(Wale et al. 2013)
	European seabass (*Dicentrarchus labrax*) exposed to filtered brown noise of varying temporal structure increased their swimming depth and group cohesion at the onset of sound exposure, but behavioural measures returned to baseline levels within the 30-min exposure period.	(Neo et al. 2014)
	European seabass (*Dicentrarchus labrax*) exposed to filtered brown noise increased their swimming depth at the onset of sound exposure, but returned to baseline behaviour within the 60-min exposure period.	(Neo et al. 2016)
	European perch *(Perca fluviatilis)* exposed to boat disturbance increased swimming activity during the first hour of exposure, but this response declined to pre-exposure levels in the next three hours of boat disturbance.	(Jacobsen et al. 2014)
Prior experience	Mountain chickadee (*Poecile gambeli*) males from characteristically noisy areas responded to white-noise playback (with a frequency spectrum of traffic noise) by singing at higher frequencies and using fewer songs from their lower bandwidth repertoire, whereas individuals from quieter areas showed opposite responses.	(LaZerte et al. 2016)
	Mountain chickadee (*Poecile gambeli*) males from characteristically noisy areas exposed to experimental noise playback (white noise with a frequency spectrum of traffic noise) switched their vocalisation type to use more songs than calls, whereas males from quiet areas used relatively more calls.	(LaZerte et al. 2017)
	Ezo brown frogs (*Rana pirica*) from characteristically noisy sites displayed faster initiation of phonotaxis and a stronger avoidance response under non-overlapping and overlapping vehicle noise respectively, compared to frogs from quiet sites.	(Senzaki et al. 2018)

	Naïve European sea bass (*Dicentrarchus labrax*) exposed to pile-driving noise had significantly elevated opercular beat rates compared to ambient-control fish **(Hedge's *g*: 0.62; CI: 0.1–1.14)**; fish exposed to 12 weeks of pile-driving noise displayed reductions in response relative to ambient-control fish **(Hedge's *g*: 0.16; CI: -0.36–0.67)**,	(Radford et al. 2016b)
	Field cricket (*Teleogryllus oceanicus*) females reared in masking traffic noise took longer to start searching and to reach a speaker simulating a singing male compared to crickets reared in silent conditions irrespective of the acoustic exposure during the testing period (masking noise, non-masking noise, silent control).	(Gurule-Small and Tinghitella 2018)
	Golden-cheeked warblers (*Setophaga chrysoparia*) from characteristically noisy sites and those from quiet sites both showed no difference in behavioural responses when exposed to playback of construction noise compared to control playbacks.	(Long et al. 2017a)
	Golden-cheeked warblers (*Setophaga chrysoparia*) from characteristically noisy sites and those from quiet sites were both more likely to respond to construction-noise playback than a silent control.	(Long et al. 2017b)
	Cynotilapia zebroides males from lower-disturbance sites displayed an increase in oxygen-consumption rate compared to ambient controls when exposed to motorboat noise **(Hedge's *g*: 0.51; CI: 0.18–0.85)**, whereas fish from higher-disturbance sites showed no difference from ambient controls **(Hedge's *g*: 0.15; CI: -0.18–0.48)**.	(Harding et al. 2018)
Multiple stressors	Damselfish (*Pomacentrus wardi*) and dottyback (*Pseudochromis fuscus*) predator–prey interactions were affected when exposed to elevated CO_2 and motorboat-noise playback both in isolation and when combined compared to ambient conditions; e.g. under present-day CO_2, predator attack speed was reduced when exposed to motorboat-noise playback compared to ambient controls **(Hedge's *g*: -0.62; CI: -1.31–0.07)**. Under high CO_2 conditions, fish exposed to noise showed no difference from ambient controls **(Hedge's *g*: -0.07; CI: -0.72–0.59)**.	(McCormick et al. 2018a)
	European sea bass (*Dicentrarchus labrax*) exposed to pile-driving noise playback under present-day CO_2 conditions had increased ventilation rates compared to ambient-sound controls under equivalent CO_2 conditions **(Hedge's *g*: 1.51; CI: 0.84–2.17)**. Under elevated CO_2 conditions, responses to noise were comparable **(Hedge's *g*: 1.69; CI: 1–2.37)**; there was no interaction between CO_2 and noise treatment.	(Poulton et al. 2016)
	Zebrafish (*Danio rerio*) exposed to white noise spent more time stationary and avoided the active speaker compared to ambient conditions, whereas dim light caused a lower number of crossings between the treatment tank and escape tank, and more time spent in the upper layer of the tank. There were no interactions between sound and light on zebrafish behaviour.	(Sabet et al. 2016b)
	Frog-biting midges (*Corethrella spp.*) were collected in acoustic traps in smaller numbers when exposed to anthropogenic noise in low light levels, whereas in high-light conditions very few midges were collected irrespective of the noise exposure.	(McMahon et al. 2017)

Table A.2. Observational studies investigating intraspecific variation in responses to anthropogenic noise as a consequence of intrinsic characteristics and extrinsic factors, as reported from the primary research papers.

Intraspecific variation	Observational Examples	References
Intrinsic characteristics		
Body size/ Age	Cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) of larger size exhibited a greater decline in abundance and catch rate than smaller individuals when there were seismic-gun surveys	(Engås et al. 1996)
	Ovenbirds (*Seiurus aurocapilla*) with less experience (were younger) were more abundant at sites near compressor stations compared to quiet areas, causing a difference in age structure between noisy and control sites	(Habib et al. 2006)
Sex	A male giant panda (*Ailuropoda melanoleuca*), but not a female, in captivity displayed behavioural responses to construction noise compared to quiet days	(Powell et al. 2006)
	A female giant panda (*Ailuropoda melanoleuca*), but not a male, in captivity displayed elevated cortisol levels on loud days compared to quiet days	(Owen et al. 2004)
Context	Killer whale (*Orcinus orca*) presence in a fjord system was negatively affected by exposure to sonar type, especially during periods of low-prey availability	(Kuningas et al. 2013)
	Bottlenose dolphins (*Tursiops truncatus*) disturbed by tour boats showed little change in maximum frequency of whistles when calves were present, but had a higher maximum frequency when calves were absent	(Heiler et al. 2016)
	Guiana dolphins (*Sotalia guianensis*) changed their whistling rate when exposed to high noise levels compared to quieter periods if they were feeding but not whilst engaged in social behaviours	(Bittencourt et al. 2017)

	Chipping sparrow (*Spizella passerina*) males whose song was characterised with lower minimum frequencies and broader bandwidths increased their minimum frequency and decreased bandwidth in response to noise, whereas those whose song had higher minimum frequencies and narrower bandwidths showed no such song adjustments in noise	(Job et al. 2016)
Repeated exposure	Harbour porpoises (*Phocoena phocoena*) showed a reduction in occurrence from exposure to a seismic airgun, although the level of response declined over the 10-day seismic survey period	(Thompson et al. 2013)
Multiple stressors	Great tit (*Parus major*) nestlings exposed to high anthropogenic noise had elevated levels of haptoglobin, whereas artificial light at night and its interaction with noise had no effect on nestling physiology	(Raap et al. 2017)
	European blackbirds (*Turdus merula*) started their dawn chorus earlier in areas with high traffic noise, although the shift was not completely separable from the effects of ambient light at night	(Nordt and Klenke 2013)
	Rufous-collared sparrows (*Zonotrichia capensis*) in areas with higher daytime noise started the dawn chorus earlier compared to areas with low anthropogenic noise, whereas light pollution levels didn't affect the onset of the dawn chorus	(Dorado-Correa et al. 2016)
	Five species of common European songbird sang earlier at dawn when occupying sites with high artificial light at night, whereas anthropogenic noise showed little effect on the timing of the dawn chorus for all species	(Da Silva et al. 2014)
	Great tit (*Parus major*) nestlings from sites characterised with varying levels of anthropogenic noise, artificial light at night, and distance from roads explained the variation in oxidative status markers	(Casasole et al. 2017)

Table A.3. Decisions to aid the design and implementation of studies considering intraspecific variation.

Decision topic	Explanation	Examples of good practice	% of studies in Table A.1
Experimental design			
Suitable controls	Suitable control treatments are required so that baseline differences between the relevant categories are established	(Neo et al. 2018)	Up to 10% did not clearly report this information
Measures of fitness	Establishing impacts on fitness directly, rather than extrapolating from short-term behavioural or physiological responses	Casper *et al.* (2013) Potvin and Macdougall-Shackleton (2015)	96% (49/51) did not measure fitness in relation to intraspecific variation
Pseudoreplication	Failure to replicate at one or more levels within an experiment (e.g. in subjects, or failure to replicate sound sources or exemplars)	(McCormick et al. 2018a)	51% (18/35) used only one real-noise source or playback exemplar
Sample size	Need sufficient sample sizes for each category being considered (e.g. males and females) rather than just the overall number of subjects	(Dorado-Correa et al. 2018)	43% (19/44) had <10 individuals per category
Captive/laboratory vs field-based experiments			
Experimental control	Laboratory experiments can be tightly controlled and isolated from confounding variables, allowing determination of underlying mechanisms, which is harder to achieve in field experiments		47% (24/51) were captive/ laboratory studies 53% (27/51) were field-based experiments No study combined both approaches
Behavioural responses	Confined animals in laboratory experiments may display a different or narrower range of behaviours. Field-experiments allow natural behaviours to be observed		
Acoustic measurements			
Full characterisation of the sound field	For aquatic studies on fish and invertebrates, this includes reporting measures from both particle-motion and sound-pressure domains. For terrestrial studies, the correct frequency weighting for the taxa needs to be applied	(LaZerte et al. 2016; LaZerte et al. 2017; Sabet *et al*. 2016b)	38% did not report detailed acoustic information (14 out of 25 fish and aquatic invertebrate studies; 3 out of 20 terrestrial studies)
Complementary use of real noise sources and loudspeaker playback	Loudspeaker playback isolates noise as the stressor, free from visual disturbances and other potential confounds. However, loudspeaker use can result in sound fields that can vary from those in real-world situations. Real noise sources are required for acoustic validity, but their use can be logistically challenging and minimising pseudoreplication is difficult. Complementary use of both methods would be best practice.	(Harding *et al*. 2018)	98% use only either loudspeaker playback or real noise sources (50 out of 51).

Appendix B: Additional material for Chapter 3

Table B.1. Global model (LMM) statistical output for the single-site opercular beat rate experiment.

Parameter (reference)	Estimate	SE	CI (95%)	t-value	df	p-value	Variance	SD
Single site: Opercular beat rate model<-lmer(OBR~Treatment*Condition+Time+(1|Trial)+(1|treat.act),data = Change)								
Sound Treatment (Motorboat)	0.93	4.77	-8.36 – 10.25	0.19	1	0.99		
Condition (Poor)	1.53	4.69	-7.63 – 10.67	0.33	1	0.86		
Time: Two	-0.61	1.17	-2.90 – 1.68	-0.52				
Time: Three	-0.84	1.17	-3.16 – 1.43	-0.72				
Time: Four	-1.40	1.18	-3.72 – 0.88	-1.20				
Time: Five	-1.36	1.18	-3.68 – 0.94	-1.16				
Treatment (Motorboat): Condition (Poor)	-1.85	6.55	-14.58 – 10.94	-0.28	1	0.78		
Random term (Trial)							280.28	16.74
Random term (Activity level)							41.94	6.48

Table B.2. Global model (GLM) statistical output for the single-site looming-stimulus experiment: startle response.

Parameter (reference)	Estimate	SE	CI (95%)	z-value	df	p-value
Single site: Looming stimulus (startle response) model_bin<-glm(Startle~Noise*Condition,data = LizardFULL, family = binomial(link = "logit"))						
Sound Treatment (Motorboat)	-1.36	0.88	-3.36 – 0.25	-1.55	1	0.25
Condition (Poor)	-1.23	0.90	-3.27 – 0.43	-1.37	1	0.40
Sound Treatment (Motorboat): Condition (Poor)	1.29	1.12	-0.84 – 3.67	1.15	1	0.24

Table B.3. Global model (GLM) statistical output for the single-site looming-stimulus experiment: distance to startle.

Parameter (reference)	Estimate	SE	CI (95%)	t-value	df	p-value
Single site: Looming stimulus (distance to startle) model<-glm(Distance.to.startle..cm.~Noise*Condition,data = Distance,family = Gamma(link = "sqrt"))						
Sound Treatment (Motorboat)	0.32	0.23	-0.12 – 0.78	1.41	1	0.16
Condition (Poor)	0.04	0.22	-0.38 – 0.47	0.16	1	0.87
Sound Treatment (Motorboat): Condition (Poor)	-0.65	0.31	-1.27 – -0.05	-2.08	1	0.04

Table B.4. Global model (LMM) statistical output for the multi-site opercular beat rate experiment.

Parameter (reference)	Estimate	SE	CI (95%)	t-value	df	p-value	Variance	SD
Multi-site: Opercular beat rate model<-lmer(Change.in.OBR~Treatment*Designation+(1|Site)+(1|Activity.Treat),data = OBR_multisite)								
Sound Treatment (Motorboat)	-4.44	2.67	-9.63 – 0.82	-1.66	1	0.83		
Designation (Healthy)	-6.35	2.75	-11.67 – -0.88	-2.31	1	0.28		
Treatment (Motorboat): Designation (Healthy)	8.23	3.82	0.68 – 15.63	2.16	1	0.03		
Random term (Site)							0.00	0.00
Random term (Activity level)							248.8	15.77

Appendix C: Additional material for Chapter 5

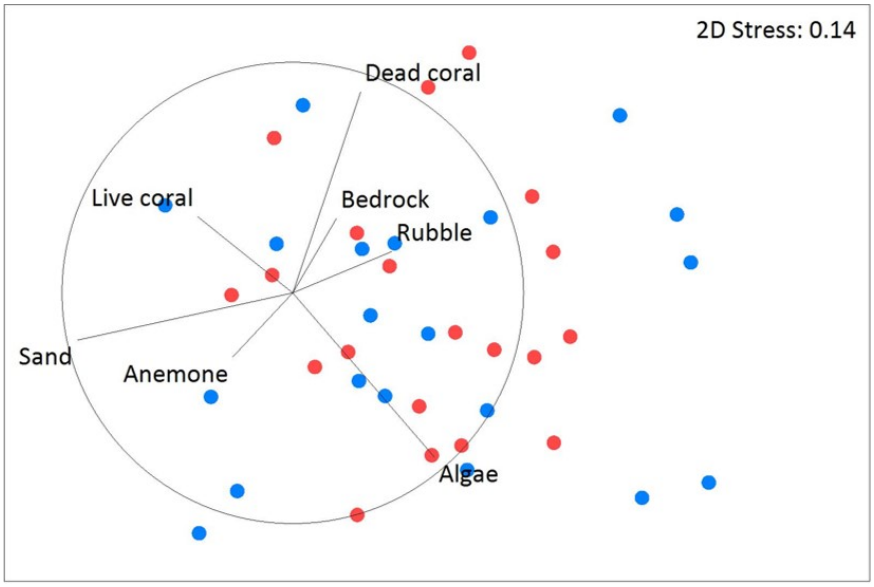

Figure C.1. Non-metric multidimensional scaling (nMDS) of benthic composition variation among survey sites of the boat channel study. Blue circles represent low-disturbance sites, red circles represent high-disturbance sites. Overlaying the nMDS plot is the contribution of substratum categories to the variation in benthic composition.

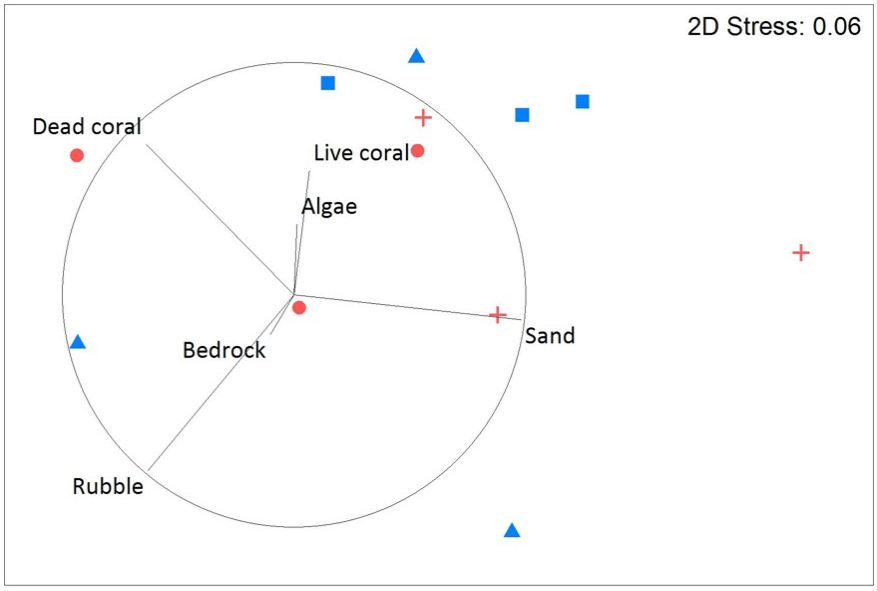

Figure C.2. Non-metric multidimensional scaling (nMDS) of benthic composition variation among motorboat manipulation sites. Colour represents manipulation treatment: ambient (blue) and motorboat (red). Symbol shape represents clusters 1 (triangle), 2 (circle), 3 (square) and 4 (cross). Overlaying the nMDS plot is the contribution of substratum categories to the variation in benthic composition.

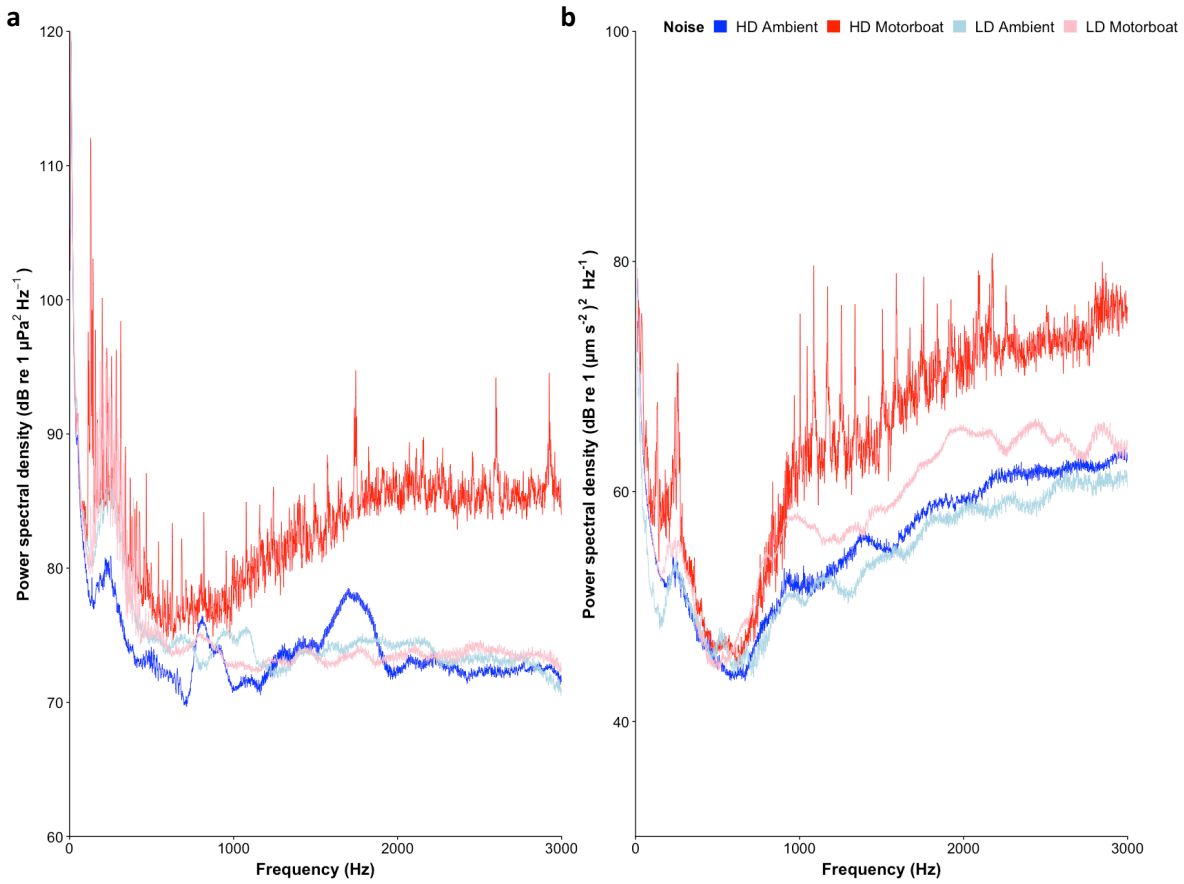

Figure C.3. Power spectral density plots of sound measurements from the boat channels prior to exclusion of fish vocalisations. All recordings analysed using *PaPAM* acoustics analysis package in MATLAB (Nedelec et al. 2016a), with a sampling rate of 44.1 kHz, Hamming window, 50% overlap, window length = sampling rate. Sound pressure (a) and monoaxial particle motion (b). Lines represent the mean power spectral density for each treatment condition.

Table C.1. SIMPER analysis of species contributions to dissimilarities between high- and low-disturbance fish community assemblages, percentage difference in fish abundance from low to high-disturbance sites and *p*-values from associated GLMMs.

Species	**Mean dissimilarity**	**SD**	**Contribution (%)**	**Cumulative (%)**	**Proportion of high- vs low-disturbance**	**GLMM *p*-value**
Stegastes nigricans	7.78	1.28	19.94	19.94	0.76	0.028
Chlorurus sordidus	7.74	1.37	19.82	39.76	1.10	0.11
Ctenochaetus striatus	2.75	1.33	7.05	46.81	1.18	0.002
Acanthurus nigrofuscus	2.67	0.66	6.84	53.65	1.11	0.53
Thalassoma hardwicke	2.33	1.06	5.98	59.63	0.73	0.0006
Scarus psitticus	2.26	1.01	5.79	65.42	0.79	0.33
Chrysiptera brownriggii	1.63	1.04	4.17	69.59	0.88	0.11
Chromis margaritifer	1.56	0.93	4.00	73.59	1.96	<0.0001
Zebrasoma scopas	0.94	1.27	2.41	76.00	1.37	0.0005
Centropyge flavissima	0.68	1.00	1.75	77.76	1.51	0.006
Halichoeres hortulanus	0.68	1.22	1.73	79.49	0.87	0.069
Abudefduf sexfasciatus	0.66	0.51	1.69	81.18	2.20	0.0006
Pseudocheilinus hexataenia	0.41	0.86	1.04	82.22	1.65	0.015
Chaetodon citrinellus	0.36	1.10	0.93	83.15	0.66	0.004
Canthigaster solandri	0.33	0.83	0.85	84.00	1.03	0.91
Halichoeres trimaculatus	0.31	0.98	0.80	84.80	0.73	0.057
Gomphosus varius	0.30	0.96	0.77	85.57	0.52	0.0001
Chaetodon lunulatus	0.28	1.02	0.71	86.28	1.17	0.39
Paracirrhites arcatus	0.28	0.88	0.70	86.99	0.68	0.15
Mulloidichthys flavolineatus	0.25	0.55	0.64	87.62	3.43	0.0003
Scarus oviceps	0.22	0.87	0.56	88.19	0.84	0.38
Stethojulis bandanensis	0.22	1.05	0.56	88.75	1.31	0.16
Dascyllus flavicaudus	0.20	0.39	0.51	89.25	0	0.0008
Balistapus undulatus	0.19	1.04	0.49	89.75	1.40	0.09

Table C.2. Global model statistical output for bite rate during the treatment and post-chronic manipulation baseline periods.

Parameter (reference)	Estimate	SE	CI (95%)	t-value	df	p-value	Variance	SD
Post-Baseline: Bite rate lmer(BiteRate~SOUND*MANIPULATION+(1|SITE),data = POST_BASELINE)								
Sound Treatment (Boat)	0.78	1.15	-1.46–3.02	0.68	1	0.49		
Manipulation (Chronic motorboat)	0.96	1.30	-1.51–3.44	0.74	1	0.47		
Sound Treatment (boat): Manipulation (Chronic motorboat)	-0.48	1.59	-3.56–2.64	-0.30	1	0.77		
Random term (SITE)							1.51	1.23
Pre-Treatment: Bite rate lmer(BiteRate~SOUND*MANIPULATION+(1|SITE),data = PRE_TREATMENT)								
Sound Treatment (Boat)	-1.83	1.11	-3.97–0.36	-1.66	1	0.09		
Manipulation (Chronic motorboat)	-0.39	1.13	-2.55–1.78	-0.34	1	0.95		
Sound Treatment (boat): Manipulation (Chronic motorboat)	1.00	1.53	-2.00–3.98	0.65	1	0.51		
Random term (SITE)							0.58	0.76
Post-Treatment: Bite rate lmer(BiteRate~SOUND*MANIPULATION+(1|SITE),data = POST_TREATMENT)								
Sound Treatment (Boat)	-1.00	1.29	-3.51–1.53	-0.78	1	0.60		
Manipulation (Chronic motorboat)	1.11	1.63	-1.98–4.25	0.68	1	0.23		
Sound Treatment (boat): Manipulation (Chronic motorboat)	1.02	1.78	-2.47–4.51	0.57	1	0.56		
Random term (SITE)							3.48	1.87

Table C.3. Global model statistical output for sheltering time during the treatment and post-baseline periods.

Parameter (reference)	Estimate	SE	CI (95%)	t-value	df	p-value	Variance	SD
Post-Baseline: Sheltering time lmer(Shelter~SOUND*MANIPULATION+(1|SITE),data = POST_BASELINE)								
Sound Treatment (Boat)	0.39	7.09	-13.36–14.06	0.06	1	0.28		
Manipulation (Chronic motorboat)	-7.39	6.64	-20.15–5.37	-1.11	1	0.50		
Sound Treatment (boat): Manipulation (Chronic motorboat)	9.04	9.75	-9.75–27.95	0.93	1	0.34		
Random term (SITE)							1.80	1.34
Pre-Treatment: Sheltering time lmer(Shelter~SOUND*MANIPULATION+(1|SITE),data = PRE_TREATMENT)								
Sound Treatment (Boat)	-0.67	6.96	-14.13–12.78	-0.10	1	0.15		
Manipulation (Chronic motorboat)	-10.00	6.51	-22.55–2.53	-1.54	1	0.46		
Sound Treatment (boat): Manipulation (Chronic motorboat)	14.14	9.58	-4.36– 32.64	1.48	1	0.13		
Random term (SITE)							1.54	1.24
Post-Treatment: Sheltering lmer(Shelter~SOUND*MANIPULATION+(1|SITE),data = POST_TREATMENT)								
Sound Treatment (Boat)	-2.66	7.51	-17.21–11.79	-0.35	1	0.38		
Manipulation (Chronic motorboat)	-11.12	7.02	-24.64–2.39	-1.58	1	0.34		
Sound Treatment (boat): Manipulation (Chronic motorboat)	13.52	10.32	-6.36–33.53	1.31	1	0.18		
Random term (SITE)							1.97	1.40

Table C.4. Global model statistical output for sediment clearing during the treatment and post-chronic manipulation baseline periods.

Parameter (reference)	Estimate	SE	CI (95%)	z-value	df	p-value	Variance	SD
Post-baseline: sediment clearing glmer(SED_binomial~SOUND*MANIPULATION+Time.in.shot..s.+(1|SITE),data = POST_BASELINE,family = binomial(link = "cauchit"))								
Sound Treatment (Boat)	-2.44	3.26	-8.83– 3.94	-0.75	1	0.15		
Manipulation (Chronic motorboat)	-2.33	2.39	-7.01– 2.35	-0.98	1	0.35		
Sound Treatment (boat): Manipulation (Chronic motorboat)	-0.35	7.48	-15.00– 14.31	-0.05	1	0.96		
Time in shot (s)	0.02	0.01	-0.01–0.05	1.24	1	0.13		
Random term (SITE)							<0.001	<0.001
Pre-Treatment: sediment clearing glmer(SED_binomial~SOUND*MANIPULATION+TIME+(1|SITE),data = PRE_TREATMENT, family = binomial(link ="cloglog"))								
Sound Treatment (Boat)	-1.55	0.80	-3.11–0.01	-1.94	1	0.21		
Manipulation (Chronic motorboat)	-1.15	0.63	-2.38–0.08	-1.84	1	0.32		
Sound Treatment (boat): Manipulation (Chronic motorboat)	1.96	1.08	-0.16–4.07	1.82	1	0.06		
Time in shot (s)	0.01	0.004	-0.001–0.02	1.65	1	0.09		
Random term (SITE)							0.00	0.00

Table C.5. Global model statistical output for conflict interactions (chasing) during the treatment and post-chronic manipulation baseline periods.

Parameter (reference)	Estimate	SE	CI (95%)	z-value	df	p-value	Variance	SD
Post-Baseline: chasing; glmer(CHASE_binomial~SOUND*MANIPULATION+TIME+(1|SITE),data = POST_BASELINE_data,family =binomial(link ="cloglog"))								
Sound Treatment (Boat)	-0.81	0.59	-1.96– 0.34	-1.38	1	0.60		
Manipulation (Chronic motorboat)	-0.76	0.53	-1.81– 0.28	-1.43	1	0.53		
Sound Treatment (boat): Manipulation (Chronic motorboat)	1.17	0.81	-0.42–2.75	1.44	1	0.15		
Time in shot (s)	0.01	0.004	0.002–0.02	2.41	1	0.01		
Random term (SITE)							<0.001	<0.001
Pre-Treatment: chasing; glmer(CHASE_binomial~SOUND*MANIPULATION+TIME+(1|SITE), data = PRE_TREATMENT, family = binomial(link = "cauchit"))								
Sound Treatment (Boat)	-0.50	1.33	-3.10–2.10	-0.38	1	0.69		
Manipulation (Chronic motorboat)	0.35	1.0	-1.60–2.30	0.35	1	0.28		
Sound Treatment (boat): Manipulation (Chronic motorboat)	1.003	1.55	-2.03–4.04	0.65	1	0.50		
Time in shot (s)	0.004	0.005	-0.01–0.01	0.73	1	0.46		
Random term (SITE)							0.43	0.66
Post-Treatment: chasing; glmer(CHASE_binomial~SOUND*MANIPULATION+TIME+(1|SITE), data = POST_TREATMENT, family = binomial(link = "probit"))								
Sound Treatment (Boat)	-0.66	0.48	-1.60–0.27	-1.39	1	0.36		
Manipulation (Chronic motorboat)	0.21	0.42	-0.61–1.03	0.50	1	0.10		
Sound Treatment (boat): Manipulation (Chronic motorboat)	0.68	0.64	-0.58–1.95	1.06	1	0.29		
Time in shot (s)	0.003	0.003	-0.002–0.008	1.23	1	0.21		
Random term (SITE)							<0.001	<0.001

Table C.6. SIMPER analysis output of species contributions to dissimilarities between the chronic-motorboat and chronic-ambient fish community assemblages, percentage difference in fish abundance from chronic-ambient to chronic-motorboat sites and *p*-values from associated GLMMs.

Species	**Mean dissimilarity**	**SD**	**Contribution (%)**	**Cumulative (%)**	**Proportion of chronic-motorboat vs chronic-ambient disturbance**	**Wilcoxon test *p*-value**
Chromis viridis	7.66	0.99	15.40	15.40	1.48	0.68
Stegastes nigricans	5.63	1.91	11.32	26.73	0.60	0.04
Chrysiptera brownriggii	4.70	0.94	9.45	36.17	2.04	0.56
Dascyllus aruanus	4.07	0.59	8.18	44.35	2.00	1
Chlorurus sordidus	3.84	1.27	7.71	52.06	0.37	0.15
Thalassoma hardwicke	3.16	1.30	6.35	58.41	1.64	0.2
Scarus psittacus	2.29	1.37	4.61	63.02	1.04	1
Halichores trimaculatus	1.95	1.36	3.92	66.95	1.58	0.42
Pseudocheilinus hexataenia	1.74	1.18	3.50	70.44	0.79	0.74
Chromis margaritifer	1.20	1.12	2.42	72.86	9.00	0.03
Ctenochaetus striatus	1.20	1.35	2.42	75.28	0.75	0.37
Halichoeres hortulanus	0.98	1.55	1.96	77.24	1.17	0.63
Canthigaster solandri	0.90	1.17	1.81	79.05	0.43	0.26
Stegastes albifasciatus	0.85	1.12	1.72	80.77	2.00	0.34
Chaetodon citrinellus	0.85	1.25	1.72	82.48	1.00	1
Centropyge flavissima	0.74	0.89	1.49	83.97	1.33	0.86
Stegastes fasciolatus	0.66	0.80	1.33	85.30	2.18	0.85
Gomphosus varius	0.50	1.21	1.00	86.30	0.52	0.40
Acanthurus nigrofuscus	0.43	1.25	0.87	87.17	0.59	0.27
Parupeneus multifasciatus	0.41	0.53	0.83	88.00	Inf	0.18
Paracirrhites arcatus	0.41	0.44	0.83	88.82	Inf	0.41
Labroides dimidiatus	0.37	0.60	0.74	89.56	2.40	1